Claim 28 was likewise discussed, and Examiner Brusca indicated his intent to enter the present amendment.

Claim 28 finds support in the specification at least at page 2, lines 10-14, as well as at page 9, lines 6-8. No new matter is added by the present amendment.

The only remaining issue in this application is the declaration of an interference between Claim 28 of the instant Horwitz application and the following Kauffman claims:

Claims 1-48 of the Kauffman '323 Patent;

Claims 1-5 of the Kauffman '192 Patent;

Claims 1-53 of the Kauffman '483 Patent;

Claims 1-46 of the Kauffman '514 Patent;

Claims 1-107 of the Kauffman '476 Patent; and

Claims 1-34 of the Kauffman '862 patent.

Applicants submitted a Request for Interference on June 9, 1999, and a Renewed Request for Interference on April 14, 2000. In response, the Examiner indicated the following:

> [I]t is the Examiner's position that the Kauffmann applications do not disclose fully random peptide sequences, because the term stochastic used and claimed by Kauffmann was never defined in the Kauffmann applications as meaning random, and the examples of stochastic sequences disclosed in the Kauffmann applications do not result in fully random sequences.

Specifically, the Examiner has interpreted the term "stochastic" to include that embodiment which results from the procedure described in the Kauffman '323 patent at Column 5, lines 26-67:

---random DNA sequence---$A_{(n)}T_{(m)}$---random DNA sequence---
---random DNA sequence---$T_{(n)}A_{(m)}$ ---random DNA sequence---

In light of the Examiner's interpretation of the term "stochastic," and in an effort to expedite the declaration of an interference, Applicants have canceled all pending claims and have added Claim 28 which is directed to a particular embodiment of the present invention, described in the specification at least at page 9, lines 6-22. This embodiment represents randomized oligonucleotides which "may alternatively include first and second randomized regions (x and z nucleotides in length, respectively) that flank on either side a linker region (y nucleotides in length) of preselected sequence." Thus, such "randomized" oligonucleotides are indeed, not fully random (i.e., the oligonucleotide "comprises" randomized sequence).

According to 37 C.F.R. §1.601(n), "[i]nvention A is the same patentable invention as an invention 'B' when invention 'A' is the same as (35 U.S.C. §102) or is obvious (35 U.S.C. §103) in view of invention 'B' assuming invention 'B' is prior art with respect to invention 'A'" [emphasis in original]. The subject matter of the Kauffman claims, using the examiner's interpretation of the term "stochastic," is clearly the same invention as the subject matter of Claim 28 of the present application.

That the linker consisting of A's bound to T's which results from the above method of Kauffman does not lend patentability to the claimed method is confirmed in the Declaration of Phillip Patten, submitted with the Response filed on June 29, 2001. Thus, the present claims are directed to the same invention as that of the above-noted Kauffman claims.

Therefore, Applicants respectfully request than an interference be declared employing the

proposed Count set forth on attached Appendix A between claims 1-48 of the Kauffman '323 Patent, claims 1-5 of the Kauffman '192 Patent, claims 1-53 of the Kauffman '483 Patent, claims 1-46 of the Kauffman '514 Patent, claims 1-107 of the Kauffman '476 Patent, claims 1-34 of the Kauffman '862 patent and claim 28 of the instant Horwitz application, all designated as corresponding to the Count. In addition, Applicants respectfully request that any pending Kauffman applications containing claims directed to the same patentable invention as defined in the Count be included in the interference.

Applicants respectfully submit that all rejections presented in the Office have now been addressed and resolved either by comment or amendment. Accordingly, Applicants respectfully request that the necessary interference now be declared. Applicants present the instant Reply in conjunction with a Second Renewed Request for Interference Pursuant to §1.607 which begins on the following page. The information required by 37 C.F.R. §1.607(a) is again set forth under headings below which correspond to the subsections of §1.607(a) to facilitate consideration by the Examiner. Because Applicants' effective filing date, July 17, 1986, is earlier than the effective filing date of the patents identified herein (November 20, 1986), the present Applicants should be declared the senior party.[1]

---

[1] Horwitz should be designated the senior party in the interference. The earliest effective filing date of Kauffman for the '192 patent is November 20, 1986, while Horwitz has an effective filing date of July 17, 1986. With respect to the effective filing dates of the other Kauffman patents, and particularly the '483, '476 and '862 patents, MPEP 2308.01 makes it clear that foreign priority under 35 U.S.C. 119 should not be taken into account in determining effective filing dates. Thus, the Kauffman claim for the benefit of the Swiss application should not be taken into account in determining senior party status. Similarly, with regard to the

I. IDENTIFICATION OF THE PATENTS AND APPLICATIONS WHICH INCLUDE SUBJECT MATTER WHICH INTERFERES WITH THE INSTANT APPLICATION

Pursuant to 37 CFR §1.607(a)(1), Applicants request that an interference be declared between the instant application and U.S. Patent Nos. 5,723,323 (the '323 patent), 5,763,192 (the '192 patent), 5,817,483 (the '483 patent), 5,824,514 (the '514 patent), 5,814,476 (the '476 patent), and 5,976,862 (the '862 patent) of Kauffman et al.[2]

II. PRESENTATION OF PROPOSED COUNT

Attached Appendix A sets forth a proposed Count pursuant to 37 CFR §1.607(a)(2). The proposed Count is an alternative Count prepared after consideration of the subject matter claimed by the respective parties.

Proposed Count A contains a representative claim of the six Kauffman Patents in that the independent claim of the '323 patent is presented in the alternative in addition to the claim of the present application. Claim 1 of the '323 patent is representative of all of the independent claims of the Kauffman patents, as discussed more fully below, and is directed to

---

Kauffman '323 and '514 patents, the filing date of the Kauffman PCT application, PCT/CH85/00099, should not be taken into account, but rather the 35 U.S.C. 102(e) date of the PCT application (November 20, 1986) because that is the date that it would be a reference against the Horwitz claims as discussed in MPEP 2308.01.

[2] Applicants note that U.S. Application Serial No. 08/464,327 of Kauffman, which was mentioned in the original Request or Interference, issued as U.S. Patent No. 5,976,862, now mentioned herein pursuant to 37 CFR §1.607.

the same patentable invention as the remaining '323 claims, the '192 claims, the '483 claims, the '514 claims, the '476 claims and the '862 claims. (See, Renewed Request for Interference, filed on April 14, 2000.)

Because applicants do not have access to the claims of the putative pending Kauffman applications, applicants were not able to copy these claims. However, it is very likely that the claims of any pending related application should also be included in the interference, given the fact that all the claims in the six issued Kauffman patents are directed to the same patentable invention as disclosed and claimed in the present application.[3] The Examiner is respectfully requested to review any related pending Kauffman applications as to whether such applications contain claims which are directed to the same patentable invention as defined by those discussed herein, and determine whether such application or applications should be included in the interference. If necessary, the Examiner is respectfully requested to suggest a claim to applicants corresponding to the claims in any pending application pursuant to 37 CFR 1.605(a) so that all related applications may be included in the interference, and amend the proposed Count.

An alternative Count which includes Kauffman Claim 1 and Claim 28 of the instant

---

[3] The '476, '483, '514 and '862 patents issued only after terminal disclaimers were filed. While the '192 patent issued without a terminal disclaimer, it is apparent from the file history that this was simply a mistake. A provisional double patenting rejection was made during prosecution of the '192 patent over copending applications 464,569 and 468,468, which have not yet issued, as well as over the applications which ultimately issued as the '476 and '483 patents. Kauffman did not contest that rejection.

application is being proposed in part because of the different language utilized by the respective parties to describe the same patentable invention.[4] For the Examiner's consideration, Appendix C is provided, in which claim 1 of the '323 patent and claim 28 of the present application are compared, element-by-element.

III. IDENTIFICATION OF CLAIMS OF THE KAUFFMAN PATENTS WHICH CORRESPOND TO THE PROPOSED COUNT PURSUANT TO 37 CFR § 1.607(a)(3)

Claims 1-48 of the Kauffman '323 Patent define the same patentable invention as claims 1-5 of the Kauffman '192 Patent, as claims 1-53 of the Kauffman '483 Patent, as claims 1-46 of the Kauffman '514 Patent, as claims 1-107 of the Kauffman '476 Patent, and as claims 1-34 of the '862 Patent. These claims correspond to and define the same patentable invention as claim 28 of the instant Horwitz application.

All the claims are essentially directed to or based on methods of searching for biologically active nucleotide sequences from among a randomly or stochastically generated population, on the theory that particular novel sequences capable of predetermined or desired biological functions may be identified. The crux of the invention reflected in all the claims rests on the discovery, which was indeed a "leap of faith" at the time the present invention was made, that randomly generated sequences could perform specific biological functions in the

---

[4] The phrase "same patentable invention" is used herein in the context of 37 CFR §1.601(n) to indicate that the inventions are the same or obvious in view of each other and should therefore be involved in an interference. The phrase should not be taken as an admission that any particular Kauffman claim is patentable under 35 U.S.C. §§ 102, 103 or 112.

absence of millions of years of evolutionary refinement. While many of the claims in the various patents and application included in this Request incorporate additional steps beyond the generation and screening of or selecting for randomly generated sequences having a particular function, such steps do not make a patentable difference when considering the state of the art at the time.

Indeed, as will be discussed herein, it was known at the time that polynucleotides could be cloned into expression vectors, and that such vectors could be transformed into and amplified in an appropriate host cell. It was known that such a host cell could be analyzed, tested or selected based on whether it expressed the protein encoded by or displayed the function provided by the cloned nucleotide sequence. It was known that a vector could then be isolated from identified transformed cells and the cloned nucleotide sequence could then be isolated from the vector using standard techniques in the art, i.e., restriction and gel purification. And it was known that such host cells could be used to produce the recombinant protein, which could be purified and used for whatever purpose it was sought, i.e., for vaccine development, as a drug, to raise antibodies, etc. Such manipulations of nucleic acids and standard recombinant techniques do not impart a patentable distinction to the novel premise at the time that a completely random population of nucleotides could generate a sequence having a particular biological activity.

Likewise, while many of the claims in the various Kauffman patents incorporate limitations which more precisely define the manner in which nucleotide sequences are

generated, or the manner in which nucleotide sequences are screened, or the particular predetermined or desired property of the nucleotide sequence identified, these specific limitations are merely variations of the general concept, and apparent applications of the method, given the state of the art at the time.

Indeed, it was known that nucleotide sequences could be synthesized chemically or by using enzymes like polymerase and terminal transferase. It was known that new nucleotide sequences could be generated by ligating smaller nucleotide sequences together. It was known that DNA could be cleaved with restriction enzymes and re-ligated using DNA ligase following hybridization of the "sticky ends" of the cleaved DNA. And it was also known that DNA could be ligated in the absence of complementary ends, i.e., blunt-end ligation.

Thus, while there are many claims which have issued in the six Kauffman patents, each merely rephrases the crux of the invention, or adds or specifies particular steps, which would have been apparent to a person skilled in the art when the basic novelty of the invention is taken in view of the state of the art. Therefore, all the claims of each Kauffman patent are believed to correspond to the Count pursuant to 37 CFR §1.607(a)(3) for the following reasons, with each patent discussed separately for the Examiner's convenience.

The Kauffman '323 Patent

The proposed Count includes Kauffman '323 claim 1 or Claim 28 of the present application joined by "or".

'323 claims 1-14

Thus, '323 claim 1 corresponds to the Count because it is included as an alternative part of the Count.

Claims 2-14 of the '323 patent all depend from '323 claim 1 and define the same patentable invention as '323 claim 1. '323 claims 2-14 therefore also correspond to the Count. For instance, claim 14 specifies that step (c) of claim 1 further comprises digesting the stochastic population of expression vectors with a restriction enzyme and religating the pieces to generate new stochastic sequences. Such a step would have been an obvious extension of the method of '323 claim 1, because the fact that new sequences could be generated by ligating together restriction fragments was well-known in the art at the time the Kauffman application was filed. See, i.e., Shortle (1983), p. 184, describing the generation of new mutant genes by digesting with restriction enzymes and inserting restriction fragments.

'323 claims 15 and 42

Kauffman '323 claim 15 corresponds to the Count because it directed to an isolated, diverse population of peptides, polypeptides or proteins comprising greater than about 1×10^5 different stochastic amino acid sequences encoded by stochastic polynucleotide sequences. This claim is obvious in view of, at the very least '323 claim 1, which is directed to a method of identifying a peptide, polypeptide or protein from a diverse population of peptides, polypeptides or proteins expressed by host cells containing stochastically generated

polynucleotide sequences. Once one has produced a population of peptides or proteins using recombinant DNA technology, one could have readily separated the peptides or proteins from the host cells to create an isolated population of diverse peptides, polypeptides or proteins using standard techniques known in the art, i.e., see Kauffman '323 col. 10, lines 13-31, discussing how purification of particular proteins and populations of proteins can be "carried out by established procedures." Hence, '323 claim 15 corresponds to the Count because it is obvious over '323 claim 1 in view of the state of the art at the time.

Similarly, '323 claim 42 corresponds to the Count because it is directed to an isolated, diverse population of peptides encoded by stochastic polynucleotide sequences (as is '323 claim 15), but specifies that the polynucleotide sequences are 300 nucleotides or less. Since limiting the length of the stochastic polynucleotide sequences would not alone present a patentable distinction, '323 claim 42 would also correspond to the Count in the same manner that claim 15 corresponds to the Count, since it is obvious in view of claim 1.

'323 claims 16-24 and 43

Kauffman '323 claim 16 corresponds to the Count because it recites a method of isolating a polynucleotide sequence following screening of host cells expressing stochastically generated polynucleotide sequences. If one could isolate a particular polynucleotide sequence from among a diverse, stochastic population, then isolating the population itself would be a clear variation, since methods for isolating a population of vectors were known at the time the

Kauffman was made. See, i.e., Maniatis et al. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York (1982), p. 2 (attached to Renewed Request). Claims 17-23 all depend from '323 claim 16 and define the same patentable invention as '323 claim 16. '323 claims 17-23 therefore also correspond to the Count.

'323 claim 24 is directed to an isolated, diverse population of polynucleotide sequences and would be obvious in view of claim 16, and therefore also corresponds to the Count, to the extent that it is obvious in view of claim 16 when viewed with the state of art at the time.

'323 claim 43 corresponds to the Count in the same manner that claim 24 corresponds to the Count. Because claim 43 is also directed to an isolated, diverse population of polynucleotide sequences which is only further defined by limiting the stochastically generated polynucleotide sequences to 300 or bases or less, and because limiting the length of the stochastic sequence does not impart a patentable distinction, claim 43 would also be obvious in view of claim 16, and therefore also corresponds to the Count as an apparent variation of claim 16.

'323 claims 25-33

Kauffman '323 claim 25 corresponds to the Count because it differs from '323 Claim 1 only in requiring isolation of the identified sequence and subsequent generation of the polypeptide, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. Claims 26-33 all

depend from '323 claim 25 and define the same patentable invention as '323 claim 25. '323 claims 26-33 therefore also correspond to the Count. For instance, '323 claim 30 specifies that the stochastically generated polynucleotide sequences of claim 25 are generated by "stochastic copolymerization." While the '323 specification does not make it clear what "stochastic polymerization" means, ligation was certainly a well known technique for joining together segments of nucleic acids at the time the Kauffman application was filed. Hence, specifying that the stochastic polynucleotide sequences were generated by "stochastic copolymerization" does not provide a patentable distinction in view of the state of the art at the time. Thus, claim 30 would also correspond to the Count, because it is directed to the same patentable invention as claim 25.

Likewise, claim 33 specifies that step (c) of the method of claim 25 further comprises digesting the vectors with a restriction enzyme and religating the pieces to form a new different population. Ligating together restriction fragments to form new sequences was well known in the art at the time the Kauffman application was filed. See, Shortle (1983) (attached to Renewed Request). Thus, claim 33 does not provide a patentable distinction over claim 25 when taken in view of the art, and would therefore also correspond to the Count.

'323 claims 34-41 and 44

'323 claim 34 corresponds to the Count because it merely recites that the synthesis is by copolymerization of A, C, G and T, which would have been obvious over '323 Claim 1 to one

of ordinary skill in the art at the time the application which issued as the '323 patent was filed. Claims 35-40 all depend from '323 claim 34 and define the same patentable invention as '323 claim 34. '323 claims 35-40 therefore also correspond to the Count, in much the same manner as claims 26-33 correspond to the Count because they are dependent on claim 25.

'323 claim 41 is directed to an isolated, diverse population of vectors comprising stochastically generated polynucleotide sequences, and is obvious in view of, at the very least claim 34, which recites a method of producing stochastically generated polynucleotide sequences by inserting them into a population of vectors. Techniques for cloning DNA sequences into vectors and methods for isolating vectors were well known techniques at the time the Kauffman application was filed, i.e., see Maniatis (1982).

'323 claim 44 would correspond to the Count in the same manner that claim 41 corresponds to the Count, since claim 44 is also directed to an isolated, diverse population of vectors which is only further defined by limiting the stochastically generated polynucleotide sequences to 300 or bases or less. Because limiting the length of the stochastic sequence does not alone impart a patentable distinction, claim 44 would also be obvious in view of claim 34, and therefore also corresponds to the Count in this manner.

Finally, claims 45-48 are product-by-process claims directed to an isolated, diverse population of peptides, polypeptides or proteins comprising stochastic amino acid sequences where the peptides are expressed from stochastically generated polynucleotide sequences, and the stochastically generated polynucleotide sequences used to express the population of

peptides are generated by copolymerization of oligonucleotides or chemical synthesis. Since both the processes of joining or ligating together oligonucleotides and chemically synthesizing oligonucleotides were well known in the art at the time the Kauffman application was filed, i.e., see Maniatis, (1982) pp. 11-14 (attached to Renewed Request), claims 45-48 would have been apparent to those of ordinary skill in the art in view of the method already set forth in '323 claim 1, which recites a method of identifying a peptide, polypeptide or protein from a stochastic population of peptides or proteins expressed from a population of stochastic polynucleotide sequences. Thus, claims 45-48 are an apparent extension of '323 claim 1, and would correspond to the Count since '323 claim 1 is part of the Count.

Thus, all of the claims of the Kauffman '323 patent correspond to the proposed Count.

The Kauffman '192 Patent

'192 claims 1-5

Kauffman '192 claim 1 corresponds to the Count because it merely contains alternative language to the '323 patent claim 1, and includes steps of isolating the polynucleotide sequence and producing a protein, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. '323 claim 1 differs from '192 claim 1 in that it defines the predetermined property of the peptide, polypeptide or protein as a "binding property to a ligand." However, such a property would have been an apparent application of the method recited in '192 claim 1, because it was known

in the art at the time that cloned nucleotide sequences could be used to produce peptides, which could be identified by virtue of a binding property. See, i.e., Matteucci and Heyneker (1983) (attached to Renewed Request), describing antibody screening of recombinant proteins; see also, the present application at page 7, lines 26-29, discussing enzyme and enzyme-substrate reactions as a method to screen for the presence of peptide.

Kauffman '192 claims 2-5 are dependent on '192 claim 1 and therefore define the same patentable invention.

'192 claims 2-5 therefore also correspond to the Count. In particular, claims 2 and 3 specify that the predetermined property of the peptide, polypeptide or protein produced by the method of '192 claim 1 has a "binding property" defined as an antigenic epitope, which may be identified by specific antibodies, respectively. Since screening by virtue of antibody reactivity was a well known technique at the time the Kauffman application was filed, i.e., see Matteucci and Heyneker (1983), describing a radioimmune assay (RIA) of transformed cells expressing recombinant protein, a peptide or protein having such a "binding property" would be an obvious extension of the method of '192 claim 1 given the state of the art at the time.

Likewise, '192 claims 4 and 5 further specify that the proteins identified using the antibody are used to make a vaccine, and more specifically, an anti-hepatitis vaccine. Using peptides and proteins to vaccinate animals against subsequent infection by the native foreign agent were well known in the art at the time the Kauffman application was filed, i.e., see McAleer et al. (1984), which describes a hepatitis vaccine made from recombinantly produced

viral protein; and Kleid et al. (1981), which describes the use of recombinantly produced foot-and-mouth disease viral protein as a vaccine (both attached to Renewed Request). Therefore, claims 4 and 5 are obvious over the method recited in '192 claim 1 in view of the state of art at the time, and would therefore also correspond to the Count.

The Kauffman '483 Patent

'483 claims 1 and 2

'483 claim 1 corresponds to the Count because it is obvious in view of '192 claim 1 when taken in view of the state of the art at the time the first Kauffman application was filed, and thus is obvious over '323 claim 1 for the same reasons '192 claim 1 is obvious over '323 claim 1. '483 claim 1 is directed to a process for the production of a peptide, polypeptide or protein identified by screening or selecting host cells carrying a library of expression vectors containing stochastically generated polynucleotide sequences, whereby the polynucleotide sequences are generated by "synthetic polynucleotide coupling." Synthetic polynucleotide coupling is a method whereby stochastic sequences may be produced by ligating stochastic oligonucleotides together to form a larger polymer. '483 Claim 1 is an apparent variation of the main concept of generating stochastic sequences because it merely adds the step of producing the polypeptide identified using the steps of '323 Claim 1, which would have been obvious to one of ordinary skill in the art. Thus, '483 claim 1 corresponds to the Count because it is an apparent variation of the method recited in claim 1 of Kauffman '192, and thus

of claim 1 of the '323 patent.

'483 claim 2 also corresponds to the Count in view of, at the very least, '192 claim 1, since the only substantive difference between the claims is that '483 claim 2 specifies that the polynucleotides produced are "at least partially" stochastic. Since "completely" is a species of "at least partially," clearly one could generate "at least partially" stochastic sequences if one knew how to generate completely stochastic sequences. Indeed, one generates "partially" stochastic nucleotide sequences when cloning a population of completely stochastic nucleotide sequences into an expression vector. Thus, '483 claim 2 would be obvious over '192 claim 1 and '483 claim 1, and therefore also over '323 claim 1, and therefore corresponds to the Count as an apparent variation of the Count.

'483 claims 3-5

'483 claims 3 and 4 are directed to methods of detecting a ligand by screening a population of peptides, polypeptides or proteins produced by stochastically generated polynucleotide sequences. Claim 3 merely differentiates over claim 4 by specifying that the stochastic polynucleotides are produced by synthetic coupling, which was a well known way to synthesize a polynucleotide sequence at the time the Kauffman application was filed as discussed above. Thus, '483 claims 3 and 4 are each merely an alternative way of looking at a method of screening a population of peptides for the ability to bind to a ligand. Thus, '483 claims 3 and 4 are apparent variations of, at the very least '192 claim 2, and would therefore

correspond to the Count in an obvious manner. In addition, since '483 claim 5 is dependent on either claim 3 or 4, it is directed to the same patentable invention and also corresponds to the Count.

'483 claims 6-16

'483 claim 6 exactly corresponds to the Count because it is directed to production of polypeptides using stochastically generated polynucleotide sequences, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. '483 claims 7-16 either directly or indirectly depend from claim 6, and therefore define the same patentable invention. Thus, claims 7-16 also correspond to the Count. For instance, translating polynucleotide sequences to produce polypeptides (as recited in '483 claims 7 and 10) was certainly known in the art. Indeed, the basic premise of recombinant DNA technology was to express nucleic acid sequences, i.e., transcribe and translate, to produce recombinantly derived proteins. Synthesizing "at least partially" stochastic sequences as recited in claims 8 and 11 would be an obvious extension of the synthesis of completely stochastic sequences as discussed above. Of course, it was also known that sequences could be cloned and "amplified" in vivo (as encompassed by '483 claim 9) by virtue of high copy number cloning vectors at the time the first Kauffman application was filed, i.e., see Muller et al. (1978), p. 345 (attached to Renewed Request). Likewise, isolating a cloned polynucleotide sequence by isolating a plasmid was also known, i.e, see id.(attached

to Renewed Request). Again, screening by binding or chemical catalysis, as recited in '483 claims 13, 15 and 16, was a well known way to screen a library at the time, as was improving a predetermined property by mutagenesis as recited in '483 claim 14 (see the instant specification at page 8, lines 4-5, discussing McClure (1985) Ann. Rev. Biochem. 54: 171-204).

'483 claims 17-28

'483 claim 17 corresponds to the Count because it is directed to production of polypeptides using stochastically generated polynucleotide sequences, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. Claims 18-28 are dependent on claim 17 in much the same manner as claims 7-16 are dependent on claim 6, which was discussed at length above. Thus, claims 18-28 also correspond to the Count in that they define the same patentable invention as '483 claim 17.

For instance, claim 24 is dependent on claim 17 and specifies that step (d) of claim 17, "producing said peptide or protein," comprises "chemical synthesis or recombinant expression." Thus, claim 24 merely states that the protein may be produced recombinantly using the polynucleotide identified in step (c), or may be synthesized chemically, presumedly based on knowledge gleaned from the nucleotide sequence of the isolated polynucleotide. However, the use of genetic information to express proteins, and chemical synthesis of

peptides based on genetic information, were both known in the art the time the Kauffman application was filed. See, i.e., "The Science Used in the Recombinant DNA Industry," Chapter 18 from Watson, Tooze and Kurtz (1983) (attached to Renewed Request). Thus, it would have been clear to those of skill in the art when faced with claim 17 of the '483 patent that the peptide, polypeptide or protein could be produced recombinantly or synthetically. Claim 24 therefore corresponds to the Count, because it is directed to the same patentable invention as claim 17 of the '483 patent.

'483 claims 29-34

'483 claim 29 corresponds to the Count because it is directed to production of a stochastic polynucleotide population, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. Claims 30-34 are dependent on claim 29 and merely specify various known ways to synthesize polynucleotide sequences. Thus, claims 30-34 define the same patentable invention as '483 claim 29, and are obvious in view of claim 29 and the state of the art at the time the Kauffman application was filed. Thus, '483 claims 30-34 also correspond to the Count.

'483 claims 35-38

'483 claims 35 and 36 correspond to the Count because they merely recite in alternative ways known uses of a peptide or protein to catalyze a reaction between two or more reactant

precursors. Given that the method of the invention permits the identification or isolation of specific peptide or protein sequences from a stochastic population by any means, including by virtue of their capability to catalyze a chemical reaction, claims 35 and 36 merely recite what would have been a standard use of a stochastic population of peptides or proteins given the state of the art. Thus, '483 claims 35 and 36 correspond to the Count because they would have been obvious over the other claims, i.e., '483 claims 6 or 17 at the least.

Claims 37 and 38 are dependent on claim 36 of the '483 patent and merely specify that the steps are repeated on a smaller population taken from the entire population of stochastic peptides, polypeptides or proteins. Thus, claims 37 and 38 define the same patentable invention, and also correspond to the Count.

'483 claims 39-53

Claims 39-53 are dependent on either claim 6, claim 17 or claim 29, each of which corresponds to the Count. In fact, claims 39-53 merely specify various sizes of populations of amino acid and polynucleotide sequences which may be employed in the methods recited in the independent claims. Since size of the stochastic library would not constitute a patentable distinction over the general method, claims 39-53 also correspond to the Count.

Thus, all of the claims in the Kauffman '483 patent correspond to the proposed Count.

The Kauffman '514 Patent

'514 claims 1-10

Claim 1 of the '514 patent corresponds to the Count because it is directed to production of an expression vector containing stochastically generated sequences, which is included within the scope of '323 claim 1. Thus, stochastic generation of polynucleotide sequences by polymerization of oligonucleotides as recited in claim 1 of the '514 patent is merely one method of synthesizing the stochastically generated polynucleotide sequences specified in step (b) of claim 1 of the '323 patent.

'514 claims 2-10 are dependent on '514 claim 1, define the same patentable invention as claim 1, and thus would also correspond to the Count. For instance, claims 3 and 7 add that the process for producing an expression vector comprising a stochastic sequence of polynucleotides as recited in '514 claim 1 includes a further step whereby the vector is cut with a restriction enzyme and religated. As discussed above, generating new sequences by ligating together restriction fragments was known at the time the Kauffman application was filed. See, i.e., Shortle (1983). Thus, claims 3 and 7 do not patentably distinguish over '514 claim 1.

'514 claims 11-12

'514 claim 11 corresponds to the Count because it merely recites a method which involves a specific way of synthesizing stochastic polynucleotide sequences. Specifically, the method requires addition of polynucleotides to the 3' end of a linearized vector with terminal transferase in order to synthesize a stochastic sequence, and a filling in of the second strand

with a polymerase enzyme, both of which were known in the art at the time the Kauffman application was filed, and would have been apparent ways to synthesize a stochastic population of polynuclectides given the concept and motivation to do so. See, i.e., Damiani et al. (1982) (attached to Renewed Request).

'514 claim 12 corresponds to the Count because it is directed to production of a library of expression vectors containing stochastically generated polynucleotide sequences, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. '514 claims 13-17 are dependent on claim 12, and some are alternatively dependent on claim 1 or 11. Yet none of claims 13-17 provides a patentable distinction over the independent claims on which they depend. In fact, claims 13, 14 and 17 are product-by-process claims, and claim 15 merely specifies that the translation product is selected from the group consisting of a peptide, polypeptide or a protein. Claim 16 indicates that the transcription product can be RNA or DNA. Given the fact that reverse transcription of RNA to generate a cDNA was known in the art at the time the Kauffman application was filed, i.e., see Rhode et al. (1981), it would have been clear to one of skill in the art that the claimed method could be applied just as easily to a stochastic population of RNA as well as DNA. Thus, claims 13-17 do not patentably distinguish over the claims on which they depend, and therefore, '514 claims 13-17 also correspond to the Count.

'514 claims 18-26

'514 claim 18 also corresponds to the Count, because, like Claim 12, it is directed to a method of producing a library or population of vectors, but claim 18 specifies that the size of the library is greater than about 1 X 10^5, and that the library may be synthesized by stochastic polymerization of double stranded oligonucleotides or nucleotide triphosphates presumedly directly on the vector, whereas claim 12 specifies that the stochastic sequences are first synthesized, then ligated into the vector. Since neither the size of the library nor the manner of synthesizing the stochastic sequences provides a patentable distinction over the general concept and motivation for doing so, claim 18 would also correspond to the Count because it is an apparent variation of claim 12. Likewise, since claims 19-26 are dependent on claim 18, they would also correspond to the Count since they define the same patentable invention as '514 claim 18, in much the same manner as several other sets of Kauffman dependent claims discussed at length above.

'514 claims 27-36

'514 claim 27 also corresponds to the Count because it is obvious over either claim 12 or claim 18 of the '514 patent since it recites a method of copolymerizing vectors containing double-stranded polynucleotides, and vectors are actually a form of polynucleotides. Hence, claim 27 corresponds to the Count also because it is obvious over claims 12 and 18 of the '514 patent. Furthermore, since claims 28-36 are dependent on claim 27 and therefore define the same patentable invention, '514 claims 28-36 also correspond to the Count.

'514 claims 37-46

'514 claim 37 corresponds to the Count because it is merely an extension of claim 18 in that it recites a method of stochastically copolymerizing double-stranded polynucleotides onto vectors which already contain stochastic or diverse polynucleotide sequences. Adding further stochastic sequences to vectors which already contain such sequences to begin with is really no different than a method of adding stochastic sequences to a vector as recited in claim 18. Thus, '514 claim 37 also corresponds to the Count in that it would have been obvious in view of claim 18. Claims 38-46 of the '514 patent are dependent on claim 37, and would therefore correspond to the Count because they define the same patentable invention as '514 claim 37.

Thus, all the claims in the Kauffman '514 patent correspond to the proposed Count.

The Kauffman '476 Patent

'476 claims 1, 2 and 8

'476 claim 1 would also correspond to the Count in view of '514 claim 12, since the only substantive difference between these claims is that '476 claim 1 adds a step of using the isolated vector or polynucleotide sequence to produce a transcription or translation product having the predetermined property. The fact that nucleic acids could be used to transcribe or translate desired products was well known in the art at the time the Kauffman application was filed. Thus, '476 claim 1 would have been an apparent extension of '514 claim 12, therefore '476 claim 1 would correspond to the Count in this manner as well.

Likewise, '476 claim 2 corresponds to the Count, because it is almost a precise duplicate of '476 claim 1, and would also correspond to the Count in an obvious manner in view of '514 claim 12. The only difference between '476 claims 1 and 2 is that claim 2 specifies that a "population" of stochastic polynucleotides is produced and inserted into a vector (rather than a single stochastic polynucleotide sequence as recited in '476 claim 1). If one isolates a "stochastic" polynucleotide sequence, by implication, the sequence must be isolated from a stochastic population. Therefore, '476 claim 2 is obvious over '476 claim 1 and '514 claim 12, and would correspond to the Count in this manner as well.

Similarly, '476 claim 8 corresponds to the Count because it is obvious in view of '476 claim 1, since a method of producing an RNA is merely a subset of a method of producing a transcription product as claimed (as discussed above for '514 claim 16). Hence, '476 claim 8 also corresponds to the Count because it is obvious in view of '476 claim 1. Since '476 claims 9-14 are dependent on either claim 1, 2 or 8, these claims would also correspond to the Count since they define the same patentable invention.

'476 claims 3-7

'476 claim 3 corresponds to the Count because it is directed to generation of stochastically generated polynucleotide sequences in an appropriate buffer, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed. Because '476 claims 4-7 are dependent on claim 3,

they would also correspond to the Count because they define the same patentable invention. In particular, claims 4-7 specify that the polynucleotide produced by the method of claim 3 has a capacity to bind to a compound, which is more specifically defined as a protein, and even more specifically defined as a protein which controls the transcription or replication of DNA. It was known in the art at the time the Kauffman application was filed that some genes contain an "enhancer" region which regulates transcription from the promoter. For instance, see the abstracts (attached to the Renewed Request) of Weiner and Botchan (1984); Zaret and Yamamoto (1984); Jost et al. (1984); and Pavvar et al. (1983), each of which describes proteins which bind to upstream enhancer elements. Similarly, see the chapter entitled "The Genetic Elements That Control Gene Expression" from Watson, Tooze and Kurtz (1983), where the action of inducers and repressors of gene expression is illustrated by reference to the lactose operon of E. coli. Thus, dependent claims 4-7 are obvious in view of independent claim 3 when viewed against the state of the art at the time, and would also correspond to the Count.

'476 claims 15-28

Likewise, '476 claim 15 corresponds to the Count because it merely specifies that the population of polynucleotides is greater than about 1×10^5 different stochastically generated polynucleotide sequences, which would have been obvious over '323 Claim 1 to one of ordinary skill in the art at the time the application which issued as the '323 patent was filed.

Because '476 claims 16-28 are dependent on claim 15, they would also correspond to the Count since they define the same patentable invention. For instance, claim 17 further specifies that the population of stochastic polynucleotide sequences is amplified. It was common knowledge in the art at the time the Kauffman application was filed that cloned DNA could be amplified in vivo using certain plasmid vectors. See, i.e., Muller et al. (1978), p. 345, which describes chloramphenicol-induced amplification of cloned DNA in a plasmid, and Maniatis, (1982) p.3.

Claims 19 and 20 are also dependent on claim 15 and specify that the claimed polynucleotides can be either RNA or DNA. It was known in the art at the time the Kauffman application was filed that RNA's could have "predetermined properties" other than the translation of proteins. For instance, see Pace and Marsh (1985) which reviews types of catalytic RNA known at the time, and Guerrier-Takada et al. (1983), which describes the catalytic activity of the RNA moiety of E. coli ribonuclease P in cleaving tRNA precursors. See also, Rhode et al. (1981), disclosing the synthesis of cDNA from RNA using reverse transcriptase. Thus, it would have been apparent to those of ordinary skill in the art when presented with '476 claim 15 that "polynucleotide" could also refer to RNA in a variety of contexts, given the many biological functions of RNA. Thus, claims 19 and 20 would correspond to the Count because they provide no patentable distinction over claim 15, on which they are dependent, and claim 15 corresponds to the Count.

Also, claim 23, as another example, is indirectly dependent on claim 15 and specifies

that the claimed method further comprises improving the predetermined property of the polynucleotide by in vitro or in vivo mutagenesis. This also would have been a well known extension of the method recited in claim 15, as discussed previously and reviewed in the present specification at page 35, lines 2-3 and page 8, lines 4-5.

'476 claims 47-60

Claim 47 would also correspond to the Count because it is obvious over '476 claim 15. The only distinction between these two claims is that '476 claim 47 adds a step of isolating the polynucleotide sequence identified as having a predetermined property. Since methods of isolating polynucleotides were well known in the art at the time the Kauffman application was filed, claim 47 of the '476 patent would have been a clear extension of claim 15. Additionally, since claims 48-60 are dependent on claim 47 in the same manner that claims 16-28 are dependent on claim 15, they would also correspond to the Count since they define the same patentable invention.

'476 claims 29-46

Similarly, claim 29 is also obvious in view of claim 15, since the only distinction is that it specifies that the predetermined property of the polynucleotide is the ability to bind a ligand. Proteins that bind to polynucleotide sequences were well known in the art at the time the Kauffman application was filed as discussed above, i.e., see Pavvar et al., which shows that

transcriptional regulatory proteins which bind to DNA were known at the latest in 1983. Furthermore, the strategy of screening a DNA library by virtue of a binding activity was also known as discussed above and evidenced by Matteucci and Heyneker (1983). Thus, claim 29 would also be obvious in view of claim 15, and also corresponds to the Count in this manner.

Additionally, because claims 30-46 are dependent on '476 claim 29, they would also correspond to the Count because they define the same patentable invention. In particular, '476 claim 41 adds a further step whereby the population of vectors is digested with a restriction enzyme such that the cloned stochastic sequences are digested, then reinserted to create new sequences. Such techniques for reassembling or creating new DNA sequences were known in the art at the time the Kauffman application was filed as discussed above, i.e., see Shortle (1983). Thus, the additional step recited in claim 41 would have been an obvious extension of the method recited in claim 29, and claim 41 would also correspond to the Count as being obvious in view of the state of the art.

'476 claims 61-78

'476 claim 61 is obvious in view of claim 29 in the same manner that claim 47 is obvious over 15. The only distinction between these two claims is that '476 claim 61 adds a step of isolating the polynucleotide sequence identified as having a predetermined property. Since methods of isolating polynucleotides were well known in the art at the time the Kauffman application was filed, claim 61 of the '476 patent would have been an obvious extension of

claim 29. Additionally, since claims 62-78 are dependent on claim 61, they would also correspond to the Count since they define the same patentable invention. In particular, claim 73 adds a restriction/ re-ligation step as discussed above for claim 41, and would be obvious over claims 61 and 29 in view of the state of the art at the time, i.e., see Shortle (1983).

'476 claims 79-90 and 103-107

Claim 79 corresponds to the Count because it is obvious over claim 1 of the '323 patent. '476 claim 79 is directed to "stochastically copolymerizing" a population of polynucleotides, which refers to the preparation of polynucleotides by joining together nucleic acid segments by processes such as ligation which were well known in the art at the time the Kauffman application was filed (see e.g. Lewin (1983), p.285, and Shortle (1983)). Thus, similar to the situation for claim 1 of the '514 patent discussed above, the generation of polynucleotide sequences by stochastic copolymerization is merely one method of synthesizing the stochastically generated polynucleotide sequences included in the scope of claim 1 of the '323 patent.

Claim 79 of the '476 patent does contain the additional limitation of producing a nucleic acid population of greater then 1×10^6 sequences. However, this limitation is not a patentably distinct limitation because it would have been obvious that the random generation of polynucleotides would result in very large numbers of polynucleotides. The reason for this is that the number of permutations increases exponentially for each randomly incorporated unit.

For example, a polynucleotide having only ten randomly incorporated bases has 4^{10} or over 10^6 random permutations. Therefore, the size of the population of polynucleotides recited in 79 would not patentably distinguish this claim over claim 1 of the '323 patent.

Thus, '476 claim 79 would correspond to the count as obvious over the other claims, particularly claim 29 of the '483 patent, in view of the state of the art. Because claims 80-90 depend from claim 79, they define the same patentable invention and therefore would also correspond to the Count. In fact, claim 83 specifies that "stochastic copolymerization" is effected by hybridization of complementary sequences. This would be accomplished by hybridization of "sticky ends" followed by ligation (see, Maniatis (1982) and Shortle (1983)). Claim 84 specifies that the stochastic polymerization occurs by ligation, which could be performed by ligation after hybridization of "sticky ends" or by blunt end ligation.

Likewise, claim 103 corresponds to the Count as being obvious over claim 79. Claim 103 is directed to an isolated population of polynucleotides having greater than about 1×10^5 different sequences. If claim 79, a method of making a stochastic population of polynucleotide sequences, corresponds to the Count, then it follows that a population of stochastic polynucleotide sequences made thereby would also correspond. Thus, '476 claim 103 would also correspond to the Count because it is obvious over the other claims, as would claims 104-107 which depend from claim 103 and define the same patentable invention.

'476 claims 91-102

Claim 91 corresponds to the count for the same reasons that claim 79 corresponds. Claim 91 is directed to stochastically polymerizing nucleic acid segments produced by cleavage, presumably by an enzyme such as a restriction endonuclease. The method of claim 91 is an obvious variation of claim 79 in which the population of polynucleotides to be stochastically copolymerized is produced by cleavage of a starting population of nucleic acids. However, production of nucleic acid segments by cleavage with endonucleases was well known in the art as evidenced by Shortle (1983) discussed above. Thus, like the situation with claim 79, the method of claim 91 is simply one method of synthesizing the stochastically generated polynucleotide sequences included within the scope of claim 1 of the '323 patent.

Like claim 79, claim 91 contains the additional limitation that a population of greater than 1 x 10^5 different sequences be produced. However, as is explained above for claim 79, this size limitation is not a patentably distinct limitation because it was obvious that stochastic copolymerization would result in very large populations of nucleic acid sequences.

Thus, claim 91 of the '476 patent also corresponds to the Count in that it is obvious over the other claims. Because claims 92-102 depend from claim 91 and define the same patentable invention, claims 92-100 would also correspond to the Count in much the same manner as the other sets of dependent claims discussed at length above.

Thus, all the Kauffman '476 claims correspond to the proposed Count.

The Kauffman '862 Patent

'862 claim 1

'862 claim 1 would also correspond to the Count in view of Kauffman '514 claim 1, for instance, because the method of '862 claim 1 is the same as '514 claim 1 except that it includes a step whereby one vector comprising a stochastic sequence is transformed into a competent host cell. Transformation techniques were well known in the art at the time that the '862 invention was made, therefore, the additional step transformation step in order to generate a transformed host cell is not a patentable distinction over '514 claim 1.

'862 claim 1 also specifies that the transformed host cell is capable of producing a transcription or translation product by virtue of the expression vector it comprises. The fact that nucleic acids could be used to transcribe or translate desired products was also well known in the art at the time the Kauffman application was filed. Thus, '862 claim 1 would have been an apparent extension of '514 claim 1, therefore '862 claim 1 would correspond to the Count in this manner as well.

'862 claim 2

'862 claim 2 would also correspond to the Count in view of '862 claim 1, because the method of '862 claim 2 has the same goal as '862 claim 1 except that it uses another means which was known in the art at the time. Specifically, whereas the method of '862 claim 1 polymerizes oligonucleotides to form stochastic sequences, the method of claim 2 uses terminal

transferase in the presence of deoxynucleotide triphosphates. Ligation of oligonucleotides and terminal transferase were both known in the art at the time as ways to generate longer nucleotide sequences as evidenced by Shortle and Damiani as discussed above. Therefore, the fact that the method of '862 claim 2 uses a different means of generating stochastic sequences than '862 claim 1 does not provide a patentable distinction. Thus, '862 claim 2 would also correspond to the Count in this manner as well.

'862 claims 3-8

'862 claim 3 would also correspond to the Count in view of '862 claims 1 and 2 taken with '483 claim 1, for instance. Indeed, the method of '862 claim 3 only differs from claims 1 and 2 of that patent in that it includes the additional steps whereby the claimed transformed host cell is isolated by a screening or selection step.

'862 claims 4-8 are dependent on claims 1-3, and do not provide any patentable distinction over the base claims in view of the state of the art at the time. In fact, claim 4 merely claims the host cell isolated by the methods of claims 1-3, and claim 5 merely rephrases the method by specifying that the transformed stochastic sequence is a template for the synthesis of a transcription or translation product, which is rather redundant in view of the preamble of claims 1-3. Claims 6 and 7 more particularly define the translation and transcription products, respectively, by specifying that the translation product may be peptide, polypeptide or protein, and the transcription product may be DNA or RNA. These are generic

categories of products which provide no patentable distinction over the general terms "transcription product" and "translation product" given the state of the art at the time. Finally, claim 8 merely specifies that the size of the library of expression vectors comprises stochastic sequences coding for at least 10,000 peptides, polypeptides or proteins. The size of the library provides no patentable distinction as discussed above, because it would have been self-evident that the random generation of polynucleotides will naturally result in very large numbers of polynucleotides, because the number of permutations increases exponentially for each randomly incorporated unit. For example, a polynucleotide comprising only ten randomly incorporated bases has 4^{10} or over 10^6 random permutations.

'862 claims 9-16

'862 claim 9 would also correspond to the Count in view of '862 claims 1-8 as a whole. For instance, like claims 1 and 2 of the '862 patent, claim 9 provides a variety of alternatives for producing stochastic sequences which are then used to produce a library of expression vectors. However, claim 9 recites a method of producing a diverse population of host cells rather than just a single host cell comprising a vector containing a stochastically-generated sequence. Because transformation protocols in general deal with a large quantity of DNA and a large population of competent cells, claiming a transformed cell in the singular or the plural is merely a matter of semantics, and does not provide any distinction in the methods employed (other than perhaps a screening or selection step to isolate a particular transformed cell).

Claims 10-16 are dependent on claim 9, and do not provide any patentable distinction given the state of the art at the time. For instance, claims 10 and 11 merely increase the size of the library of stochastic sequences. As discussed above, the fact that the size of the library will increase with the length of the random sequences is self-evident, and does not provide a patentable distinction in the absence of unexpected results, difficulties or advantages (none of which is disclosed in Kauffman '862). The fact that claim 15 claims "at least partially stochastic" sequences is not distinctive as discussed above because, if one can generate fully stochastic sequences, one can also generate partially stochastic sequences and indeed does so when cloning a fully stochastic sequence into a cloning vector. Claim 16 provides a further step whereby the ligated vectors are digested with a restriction enzyme that only cuts within the stochastic insert portion, and the vectors are re-ligated to generate new sequences. Such techniques for reassembling or creating new DNA sequences were known in the art at the time the Kauffman application was filed as discussed above, i.e., see Shortle (1983). Thus, none of the dependent claims 10-16 provide a patentable distinction over claim 9, and would therefore be covered by the proposed Count to the extent that claim 9 is covered given the state of the art at the time.

'862 claims 17-25

'862 claim 17 would also correspond to the Count because it merely re-phrases in broader terms the method recited in '862 claim 9. In like manner, claims 18-24 merely repeat

the limitations of claims 10-14 with regard to the size of the library and the manner in which stochastic sequences may be generated. And claim 25 is merely an extended abstraction by reciting that the claimed diverse population of vectors comprises two or more diverse populations of vectors. How many diverse populations are contained within a single diverse population merely depends on how many ways one can envision separating the members of a population according to different characteristics.

'862 claims 26-34

Finally, '862 claim 26 would also correspond to the Count due to the fact that it also merely re-phrases in broader terms the method recited in '862 claim 9 (which in turn corresponds to the Count for the reasons provided above), and also includes the concept recited in claim 16 discussed above whereby vectors with stochastic sequences are digested with restriction enzymes and ligated back together. As this concept does not provide a patentable distinction over other methods of generating stochastic sequences given the state of the art at the time, i.e., Shortle (1983), claim 26 would also correspond to the Count by virtue of its similarity to the claims discussed above. Claims 27-34 again repeat the limitations of claims 18-25 except that they are dependent on claim 26 rather than claim 17. Nevertheless, they fail to provide a patentable distinction over independent claim 26 for the same reasons discussed above, and would therefore also correspond to the Count.

Thus, all the claims in the Kauffman '862 patent correspond to the proposed Count.

IV. CLAIM OF THE HORWITZ APPLICATION WHICH CORRESPONDS TO THE PROPOSED COUNT PURSUANT TO 37 CFR §1.607(a)(4)

As discussed in the original Request for Interference filed on June 9, 1999, Claim 28 of the instant application is believed to correspond to the proposed Count. The proposed Count includes '323 Claim 1 or Claim 28 of the present application, as discussed above. Claim 28 thus corresponds exactly to one part of the proposed Count. Because of the use of "or," correspondence to one part of the proposed Count is sufficient.

V. EXPLANATION OF HOW THE REQUIREMENT OF 35 U.S.C. §135(b) IS MET

According to 35 U.S.C. §135(b), "[a] claim which is the same as, or for the same or substantially the same subject matter as, a claim of an issued patent may not be made in any application unless such a claim is made prior to one year from the date on which the patent was granted." In the instant case, the Kauffman '323 Patent issued on March 3, 1998. Claims 3, 4, 6-8 and 11-14 of the instant application were filed in a preliminary amendment in the instant application on March 3, 1999. These claims, although canceled by the present amendment, were for "the same as, or for the same or substantially the same subject matter as" claims 1, 16, 24, 25, 34 and 41 of the Kauffman '323 patent, and were present prior to one year from the date on which the Kauffman '323 Patent issued.

Likewise, the Kauffman '192 Patent issued on June 9, 1998. Claim 15 of the instant

application was submitted in the Supplemental Preliminary Amendment filed on June 9, 1999. Claim 15, although canceled by the present amendment, was for "the same as, or for the same or substantially the same subject matter as" the claim 1 of the Kauffman '192 patent, and was present prior to one year from the date on which this patent issued.

The Kauffman '483 patent issued on October 6, 1998. Claims 16-20 of the instant application were submitted in the Supplemental Preliminary Amendment filed on June 9, 1999. Claims 16-20, although canceled by the present amendment, were for "the same as, or for the same or substantially the same subject matter as" claims 6, 17, 29, 35 and 36, respectively, of the Kauffman '483 patent, and were present prior to one year from the date on which this patent issued.

The Kauffman '514 patent issued on October 20, 1998. Claims 21-23 of the instant application were submitted in the Supplemental Preliminary Amendment filed on June 9, 1999. Claims 21-23, although canceled by the present amendment, were for "the same as, or for the same or substantially the same subject matter as" claims 1, 12 and 18, respectively, of the Kauffman '514 patent, and were present prior to one year from the date on which this patent issued.

The Kauffman '479 patent issued on September 29, 1998. Claim 24 was submitted in the Supplemental Preliminary Amendment filed on June 9, 1999. Claim 24, although canceled by the present amendment, was directed to "the same as, or for the same or substantially the same subject matter as" claims 3 and 15, respectively, of the Kauffman '476 patent, and was

present prior to one year from the date on which this patent issued.

The Kauffman '862 patent issued on November 2, 1999. Claims 26-27 were submitted in the Reply to Office Action filed above on this date, January 14, 2000. Claims 26-27, although canceled by the present amendment, were directed to "the same as, or for the same or substantially the same subject matter as" claims 1 and 2, respectively, of the Kauffman '862 patent, and were present prior to one year from the date on which this patent issued.

VI. EXPLANATION OF WHY AN INTERFERENCE SHOULD BE DECLARED

As stated in 37 C.F.R. §1.601(i), "[a]n interference is a proceeding instituted in the Patent and Trademark Office before the Board to determine any question of patentability and priority of invention between two or more parties claiming the same patentable invention" [emphasis in original]. According to 37 C.F.R. §1.601(n), "[i]nvention A is the same patentable invention as an invention 'B' when invention 'A' is the same as (35 U.S.C. §102) or is obvious (35 U.S.C. §103) in view of invention 'B' assuming invention 'B' is prior art with respect to invention 'A'" [emphasis in original].

Claims 1-48 of the Kauffman '323 Patent define the same patentable invention as claims 1-5 of the Kauffman '192 Patent, as claims 1-53 of the Kauffman '483 Patent, as claims 1-46 of the Kauffman '514 Patent, as claims 1-107 of the Kauffman '476 Patent, as claims 1-34 of the Kauffman '862 patent, and as claim 28 of the instant Horwitz application. All the claims are essentially directed to or based on methods of searching for biologically active nucleotide

sequences from among a randomly or stochastically generated population, on the theory that particular novel sequences capable of predetermined or desired biological functions may be identified. The crux of the invention reflected in all the claims rests on the discovery, which was indeed a "leap of faith" at the time the present invention was made, that randomly generated sequences could perform specific biological functions in the absence of millions of years of evolutionary refinement. While many of the claims in the various Kauffman patents incorporate additional steps beyond the generation and screening of or selecting for randomly generated sequences having a particular function, such steps do not make a patentable difference when considering the state of the art at the time.

Indeed, it was known at the time that polynucleotides could be cloned into expression vectors, and that such vectors could be transformed into and amplified in an appropriate host cell. It was known that such a host cell could be analyzed, tested or selected based on whether it expressed the protein encoded by or displayed the function provided by the cloned nucleotide sequence. It was known that vector could then be isolated from identified transformed cells and the cloned nucleotide sequence could then be isolated from the vector using standard techniques in the art, i.e., restriction and gel purification. And it was known that such host cells could be used to produce the recombinant protein, which could be purified and used for whatever purpose it was sought, i.e., for vaccine development, as a drug, to raise antibodies, etc. Such manipulations of nucleic acids and standard recombinant techniques do not generally impart a patentable distinction to the novel premise at the time that a completely random

population of nucleotides could generate a sequence having a particular biological activity.

Likewise, while many of the claims in the various Kauffman patents incorporate limitations which more precisely define the manner in which nucleotide sequences are generated, or the manner in which nucleotide sequences are screened, or the particular predetermined or desired property of the nucleotide sequence identified, these specific limitations are merely variations of the general concept, and apparent applications of the method, given the state of the art at the time.

Indeed, it was known that nucleotide sequences could be synthesized chemically or by using enzymes like polymerase and terminal transferase. It was known that new nucleotide sequences could be generated by ligating smaller nucleotide sequences together. It was known that DNA could be cleaved with restriction enzymes and re-ligated using DNA ligase following hybridization of the "sticky ends" of the cleaved DNA. And it was also known that DNA could be ligated in the absence of complementary ends, i.e., blunt-end ligation.

Thus, while there are many claims which have issued in the six Kauffman patents, each merely rephrases the crux of the invention, or adds or specifies particular steps, which would have been apparent to a person skilled in the art when the basic novelty of the invention is taken in view of the state of the art. Furthermore, while the language of the Kauffman claims is slightly different than Horwitz claim 28, all of the claims are based on the same novel premise: that novel nucleotide sequences may be identified in a random population which have

or exhibit a desired biological activity.[5]

Therefore, because the Kauffman and Horwitz claims define the same patentable invention, an interference between claims 1-48 of the Kauffman '323 Patent, claims 1-5 of the Kauffman '192 Patent, claims 1-53 of the Kauffman '483 Patent, claims 1-46 of the Kauffman '514 Patent, claims 1-107 of the Kauffman '476 Patent, claims 1-34 of the Kauffman '862 patent and claim 28 of the instant Horwitz application should be declared.

With regard to the Kauffman applications which are thought to be still pending at the Patent & Trademark Office, while applicants do not have specific knowledge of any claim in such applications, given the fact that all the claims in the six issued Kauffman patents are directed to the same patentable invention as disclosed and claimed in the present application, applicants suspect that any pending Kauffman application should also be included in the interference. Indeed, in view of the many ways in which Kauffman has defined the same invention through the claims of the six issued patents, it is expected that Kauffman will repackage the invention in as many ways as are linguistically possible in any applications which remain pending.

The Examiner is respectfully requested to review any related pending application as to

---

[5] Random, "without reference to a wild type sequence," emphasizes the manner in which the present invention distinguishes over the prior art. Oligonucleotides for the typical mutagenesis experiment of the prior art were synthesized with a bias toward wild type. In contrast, the present invention requires no prior knowledge of structure-function relationships. However, this language does not preclude situations where there is knowledge of a wild type sequence. Rather, it merely indicates that such knowledge is not required.

whether such application contains claims which are directed to the same patentable invention as defined by those discussed herein, and determine whether such application or applications should be included in the interference. If necessary, the Examiner is respectfully requested to suggest a claim to applicants corresponding to the claims in any pending application pursuant to 37 CFR 1.605(a) so that all related applications may be included in the interference.

VII. CONCLUSION

Applicants respectfully request than an interference be declared employing the proposed Count set forth on attached Appendix A between claims 1-48 of the Kauffman '323 Patent, claims 1-5 of the Kauffman '192 Patent, claims 1-53 of the Kauffman '483 Patent, claims 1-46 of the Kauffman '514 Patent, claims 1-107 of the Kauffman '476 Patent, claims 1-34 of the Kauffman '862 patent and claim 28 of the instant Horwitz application, all designated as corresponding to the Count. In addition, Applicants respectfully request that any pending Kauffman applications containing claims directed to the same patentable invention as defined in the Count be included in the interference. Such action is respectfully requested.

Respectfully submitted,

Burns, Doane, Swecker & Mathis, L.L.P.

By: R. Danny Huntington

R. Danny Huntington
Registration No. 27,903
Sharon E. Crane, Ph.D.
Registration No. 36,113

P.O. Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: August 9, 2001

APPENDIX A

PROPOSED COUNT A

Claim 1 of the '323 patent

1. A method of identifying a peptide, polypeptide or protein having a binding property to a ligand, comprising:

(a) providing a ligand for detecting said binding property;

(b) synthesizing a diverse population of stochastically generated polynucleotide sequences;

(c) inserting said diverse population of stochastically generated polynucleotide sequences into a population of expression vectors to form a diverse population of expression vectors containing stochastically generated polynucleotide sequences;

(d) expressing in host cells said diverse population of expression vectors containing stochastically generated polynucleotide sequences to produce a diverse population of peptides, polypeptides or proteins; and

(e) screening said diverse population of peptides, polypeptides or proteins with said ligand under conditions which allow binding and detection of one or more peptides, polypeptides or proteins having said predetermined property.

or

Claim 28 of the '231 application

28. A method of identifying a functional nucleotide sequence which provides a desired biological activity comprising:

a. providing a means for detecting said desired biological activity;

b. synthesizing by enzymatic or chemical synthesis a mixed population of nucleotide sequences,

wherein said nucleotide sequences comprise an oligonucleotide consisting of a 5' randomized, a central preselected sequence and a 3' randomized sequence, and wherein said randomized sequences are synthesized without reference to a wild type sequence;

c. introducing a plurality of the nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing the nucleotide sequences;

d. introducing said cloning vectors into suitable host cells;

e. expressing said cloning vectors in said host cells; and

f. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors which comprise a functional nucleotide sequence which provides the desired biological activity.

Claims of U.S. Patent No. 5,723,323

APPENDIX B

Kauffman claims

5,723,323

1. A method of identifying a peptide, polypeptide or protein having a binding property to a ligand, comprising:

(a) providing a ligand for detecting said binding property;

(b) synthesizing a diverse population of stochastically generated polynucleotide sequences;

(c) inserting said diverse population of stochastically generated polynucleotide sequences into a population of expression vectors to form a diverse population of expression vectors containing stochastically generated polynucleotide sequences;

(d) expressing in host cells said diverse population of expression vectors containing stochastically generated polynucleotide sequences to produce a diverse population of peptides, polypeptides or proteins; and

(e) screening said diverse population of peptides, polypeptides or proteins with said ligand under conditions which allow binding and detection of one or more peptides, polypeptides or proteins having said predetermined property.

2. The method of claim 1, wherein said stochastically generated polynucleotide sequences further comprises all twenty amino acid residues encoded at each codon position.

3. The method of claim 1, wherein said diverse population of stochastically generated polynucleotide sequences, encode at least 10,000 different peptides, polypeptides or proteins.

4. The method of claim 1, wherein said inserting further comprises hybridization of complementary ends.

5. The method of claim 1, wherein said inserting further comprises ligation.

6. The method of claim 1, wherein said diverse population of stochastically generated polynucleotide sequences are produced by stochastic copolymerization of double stranded oligonucleotides.

7. The method of claim 1, wherein said diverse population of stochastically generated polynucleotide sequences are produced by copolymerization of the four kinds of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine and Thymine.

8. The method of claim 1, wherein said diverse population of stochastically generated polynucleotide sequences are produced by chemical synthesis.

9. The method of claim 1, wherein the expression vector is a plasmid.

10. The method of claim 9, wherein the plasmid is pUC8.

11. The method of claim 1, wherein the expression vector is vital DNA.

12. The method of claim 1, wherein the expression vector is a hybrid of plasmid and vital DNA.

13. The method of claim 1, wherein the expression vector is a phage.

14. The method of claim 1, wherein step (c) further comprises digesting the diverse population of expression vectors with a restriction enzyme having a recognition sequence absent in the expression vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of new stochastic polynucleotide sequences.

15. An isolated, diverse population of peptides, polypeptides, or proteins comprising greater than about 1×10^5 different stochastic amino acid sequences encoded by stochastic polynucleotide sequences.

16. A method of isolating a polynucleotide sequence encoding a peptide, polypeptide or protein having a predetermined binding property to a ligand, comprising:

(a) providing a ligand for detecting said binding property;

(b) synthesizing a diverse population of stochastically generated polynucleotide sequences;

(c) inserting said diverse population of stochastically generated polynucleotide sequences into a population of expression vectors to form a diverse population of expression vectors containing stochastically generated polynucleotide sequences;

(d) expressing in host cells said diverse population of expression vectors containing stochastically generated polynucleotide sequences to produce a diverse population of peptides, polypeptides or proteins;

(e) screening said diverse population of peptides, polypeptides or proteins with said ligand under conditions which allow binding and detection of one or more peptides, polypeptides or proteins to said ligand; and

(f) isolating the stochastically generated polynucleotide sequence or sequences which encoding said peptides, polypeptides or proteins having said predetermined binding property to said ligand.

17. The method of claim 16, wherein said stochastically generated polynucleotide sequences further comprises all twenty amino acid residues encoded at each codon position.

18. The method of claim 16, wherein said diverse population of stochastically generated polynucleotide sequences, encode at least 10,000 different peptides, polypeptides or proteins.

19. The method of claim 16, wherein said inserting further comprises hybridization of complementary ends.

20. The method of claim 16, wherein said inserting further comprises ligation.

21. The method of claim 16, wherein said diverse population of stochastically generated polynucleotide sequences are produced by stochastic copolymerization of double stranded oligonucleotides.

22. The method of claim 16, wherein said diverse population of stochastically generated polynucleotide sequences are produced by copolymerization of the four kinds of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine and Thymine.

23. The method of claim 16, wherein step (c) further comprises digesting the diverse population of expression vectors with a restriction enzyme having a recognition sequence absent in the expression vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of new stochastic

polynucleotide sequences.

24. An isolated, diverse population of polynucleotide sequences which encode a diverse population of ligand binding peptides, polypeptides, or proteins comprising greater than about 1×10^5 different stochastic amino acid sequences encoded by the stochastic polynucleotide sequences.

25. A method of isolating a peptide, polypeptide or protein having a binding property, comprising:

(a), providing a ligand for detecting said binding property;

(b) synthesizing a diverse population of stochastically generated polynucleotide sequences;

(c) inserting said diverse population of stochastically generated polynucleotide sequences into a population of expression vectors to form a diverse population of expression vectors containing stochastically generated polynucleotide sequences;

(d) expressing in host cells said diverse population of expression vectors containing stochastically generated polynucleotide sequences to produce a diverse population of peptides, polypeptides or proteins;

(e) screening said diverse population of peptides, polypeptides or proteins with said ligand under conditions which allow binding and detection of one or more peptides, polypeptides or proteins having said predetermined property;

(f) isolating the stochastically generated polynucleotide sequence or sequences encoding said peptides, polypeptides or proteins having said binding property to said ligand; and

(g) using genetic information from said isolated stochastically generated polynucleotide sequence to produce said peptide, polypeptide or protein having said binding property.

26. The method of claim 25, wherein said stochastically generated polynucleotide sequences further comprises all twenty amino acid residues encoded at each codon position.

27. The method of claim 25, which said diverse population of stochastically generated polynucleotide sequences, encode at least 10,000 different peptides, polypeptides or proteins.

28. The method of claim 25, wherein said inserting further comprises hybridization of complementary ends.

29. The method of claim 25, wherein said inserting further comprises ligation.

30. The method of claim 25, wherein said diverse population of stochastically generated polynucleotide sequences are produced by stochastic copolymerization of double stranded oligonucleotides.

31. The method of claim 25, wherein said diverse population of stochastically generated polynucleotide sequences are produced by copolymerization of the four kinds of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine and Thymine.

32. The method of claim 25, wherein said diverse population of stochastically generated polynucleotide sequences are produced by chemical synthesis.

33. The method of claim 25, wherein step (c) further comprises digesting the diverse population of expression vectors with a restriction enzyme having a recognition sequence

absent in the expression vector and reinserting the digested products into said digested population of vectors to form a new different population having a greater number of new stochastic polynucleotide sequences.

34. A method of producing a diverse population of stochastically generated polynucleotide sequences encoding a diverse population of ligand binding peptides, polypeptides or proteins, comprising:

(a) synthesizing a diverse population of stochastically generated polynucleotide sequences selected from a method consisting of stochastic copolymerization of double stranded oligonucleotides, copolymerization of the four kinds of nucleotide triphoshpates consisting of Adenine, Cytosine, Guanine and Thymine, and chemical synthesis; and

(b) inserting said diverse population of stochastically generated polynucleotide sequences into a population of vectors to form a diverse population of vectors containing stochastically generated polynucleotide sequences.

35. The method of claim 34, wherein said stochastically generated polynucleotide sequences further comprises all twenty amino acid residues encoded at each codon position.

36. The method of claim 34, which said diverse population of stochastically generated polynucleotide sequences, encode at least 10,000 different peptides, polypeptides or proteins.

37. The method of claim 34, wherein said inserting further comprises hybridization of complementary ends.

38. The method of claim 34, wherein said inserting further comprises ligation.

39. The method of claim 34, further comprising introducing said diverse population of vectors containing stochastically generated polynucleotide sequences into host cells.

40. The method of claim 34, wherein step (b) further comprises digesting the diverse population of expression vectors with a restriction enzyme having a recognition sequence absent in the expression vector and reinserting the digested products into said digested population of vectors to form a new different population having a greater number of new stochastic polynucleotide sequences.

41. An isolated, diverse population of vectors comprising stochastically generated polynucleotide sequences encoding a diverse population of ligand binding peptides, polypeptides, or proteins comprising greater than about 1×10^5 different stochastic amino acid sequences.

42. An isolated, diverse population of peptides or polypeptides comprising stochastic amino acid sequences encoded by stochastic polynucleotide sequences of about 300 nucleotides or less in length.

43. An isolated, diverse population of polynucleotides sequences encoding a diverse population of ligand binding peptides or polypeptides comprising stochastic amino acid sequences encoded by stochastic polynucleotide sequences of about 300 nucleotides or less in length.

44. An isolated, diverse population of vectors encoding a diverse population of ligand binding peptides or polypeptides comprising stochastic amino acid sequences encoded by stochastically generated polynucleotide sequences of about 300 nucleotides or less in length.

45. An isolated, diverse population of peptides, polypeptides, or proteins comprising stochastic amino acid sequences produced by a method comprising synthesizing a diverse population of

stochastically generated polynucleotide sequences selected from a method consisting of stochastic copolymerization of double stranded oligonucleotides, copolymerization of the four kinds of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine and Thymine, and chemical synthesis; and expressing said stochastically generated polynucleotide sequences.

46. The isolated diverse population of peptides, polypeptides, or proteins of claim 15, further comprising greater than about 1×10^6 different stochastic amino acid sequences.

47. The isolated diverse population of peptides, polypeptides, or proteins of claim 15, further comprising greater than about 1×10^7 different stochastic amino acid sequences.

48. The isolated, diverse population of peptides, polypeptides, or proteins of claim 15, further comprising greater than about 1×10^8 different stochastic amino acid sequences.

5,763,192

1. A process for the production of a peptide, polypeptide, or protein having a predetermined property, comprising the steps of:

producing by enzymatic or chemical coupling, stochastically generated polynucleotide sequences;

forming a library of expression vectors containing such stochastically generated polynucleotide sequences;

culturing host cells containing the vectors to produce peptides, polypeptides, or proteins encoded by the stochastically generated polynucleotide sequences;

carrying out screening or selection on such host cells, to identify a peptide, polypeptide, or protein produced by the host cells having the predetermined property;

isolating a stochastically generated polynucleotide sequence which encodes the identified peptide, polypeptide, or protein;

using the isolated sequence to produce the peptide, polypeptide, or protein having the predetermined property.

2. The process of claim 1, wherein said peptide, polypeptide or protein having said binding property comprises at least one epitope similar to an amino acid sequence of one of the epitopes of a given antigen.

3. The process of claim 1, wherein said peptide, polypeptide or protein having said binding

property comprises stimulating or modifying the effects of a biologically active molecule, and screening and/or selection of the clones of transformed host cells producing at least one peptide or polypeptide having this property is carried out by preparing antibodies against that molecule, and utilizing these antibodies so obtained to identify those clones containing those peptides or polypeptides, then by growing the clones thus identified and separating and purifying the peptide or polypeptide produced by these clones, and finally by submitting these peptide(s) or polypeptide(s) to an assay in vitro to verify that it has in fact the capacity to simulate or modify the effects of the said molecule.

4. The process of claims 2 or 3 for the preparation of a vaccine, said process comprising antibodies against a pathogenic agent that are obtained and used to identify those clones producing at least one protein having at least one epitope similar to an amino acid sequence of one of the epitopes of the pathogenic agent, that the corresponding clones of transformed host cells are grown in such a manner as to produce this protein, that the protein is isolated and purified from the cultures of clones of cells and that this protein is used for the production of a vaccine against the pathogenic agent.

5. The process of claim 4 for the preparation of an anti-hepatitis B virus vaccine, said process comprising that at least one capsid protein of the hepatitis B virus is extracted and purified, that this protein is injected into the body of an animal capable of forming antibodies against this protein, that these antibodies are recovered and purified, that these antibodies are used to identify those clones producing at least one protein having at least one epitope similar to one of the epitopes of the hepatitis B virus, that the clones of transformed host cells corresponding to these clones are grown in a manner to produce this protein, that this protein is isolated and purified from these cultures of host cells, and that this protein is used for the production of an anti-hepatitis B virus vaccine.

5,817,483

1. A process for the production of a peptide, polypeptide, or protein having a predetermined property, comprising the steps of:

producing by synthetic polynucleotide coupling, a population of stochastically generated polynucleotide sequences;

forming a library of expression vectors containing said population of stochastically generated polynucleotide sequences;

introducing the vectors into host cells;

culturing the host cells;

carrying out screening or selection on said host cells, to identify a peptide, polypeptide, or protein produced by the host cells having the predetermined property;

isolating a stochastically generated polynucleotide sequence which encodes the identified peptide, polypeptide, or protein;

using the isolated sequence to produce the peptide, polypeptide, or protein having the predetermined property.

2. A process for the production of a peptide, polypeptide, or protein having a predetermined property, comprising the steps of:

producing a population of at least partially stochastic synthetic polynucleotide sequences;

introducing the population of at least partially stochastic polynucleotide sequences into host cells to produce transformed host cells;

cultivating the transformed host cells to produce peptides, polypeptides, or proteins expressed by at least some of the stochastic polynucleotide sequences;

carrying out screening and/or selection methods on said transformed host cells to identify clones producing the peptide, polypeptide, or protein having the predetermined property;

isolating the clones so identified; and

growing the isolated clones in a manner so as to produce the peptide, polypeptide, or protein having the predetermined property.

3. A process for the detection or titration of a ligand using a peptide, polypeptide, or protein having a predetermined property, comprising the steps of:

producing by synthetic polynucleotide coupling, a population of stochastically generated polynucleotide sequences;

forming a library of expression vectors containing said population of stochastically generated polynucleotide sequences;

introducing the vectors into host cells;

culturing the host cells containing the vectors to produce peptides, polypeptides, or proteins encoded by the stochastically generated polynucleotide sequences;

carrying out screening or selection on said host cells, to identify a peptide, polypeptide, or protein produced by the host cells having the predetermined property;

contacting the peptide, polypeptide, or protein with two or more concentrations of a ligand; and

determining the amount of peptide, polypeptide or protein bound at each concentration of ligand.

4. A process for the detection or titration of a ligand using a peptide, polypeptide, or protein having a predetermined property, comprising the steps of:

producing a population of at least partially stochastic synthetic polynucleotide sequences;

introducing the population of at least partially stochastic polynucleotide sequences into host cells to produce transformed host cells;

cultivating the transformed host cells to produce peptides, polypeptides, or proteins expressed by at least some of the stochastic polynucleotide sequences;

carrying out screening and/or selection methods on said transformed host cells to identify clones producing the peptide, polypeptide, or protein having the predetermined property;

contacting the peptide, polypeptide, or protein with two or more concentrations of a ligand; and

determining the amount of peptide, polypeptide or protein bound at each concentration of

ligand.

5. The process according to claim 3 or claim 4, wherein more than one ligand is detected or titrated.

6. A method of identifying a peptide, polypeptide or protein having a predetermined property, comprising:

(a) producing a population of peptides, polypeptides or proteins encoded by stochastic polynucleotide sequences;

(b) screening said population of peptides, polypeptides or proteins for said predetermined property under conditions which allow detection of one or more peptides, polypeptides or proteins having said predetermined property.

7. The method of claim 6, wherein step (a) further comprises synthesizing a population of stochastic polynucleotide sequences and translating said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

8. The method of claim 6, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences and translating said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

9. The method of claim 7 or 8, further comprising amplification of said population of stochastic polynucleotide sequences.

10. The method of claim 6, wherein step (a) further comprises synthesizing a population of stochastic polynucleotide sequences and expressing said population of stochastic polynucleotide

sequences to produce said population of peptides, polypeptides or proteins.

11. The method of claim 6, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences and expressing said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

12. The method of claim 6, further comprising isolating the polynucleotide sequence encoding said one or more peptides, polypeptides or proteins having said predetermined property.

13. The method of claim 6, wherein said predetermined property comprises binding or chemical catalysis.

14. The method of claim 13, further comprising improving said predetermined property by in vitro or in vivo mutagenesis.

15. The method of claim 13, wherein said binding further comprises modification of a biological or chemical property of a compound bound by said peptide, polypeptide or protein.

16. The method of claim 15, wherein said modification of said biological or chemical property of said compound further comprises stimulating or inhibiting at least one biological function of said compound.

17. A method of producing a peptide, polypeptide or protein having a predetermined property, comprising:

(a) producing a population of peptides, polypeptides or proteins encoded by stochastic polynucleotide sequences;

(b) screening said population of peptides, polypeptides or proteins for said predetermined property under conditions which allow detection of one or more peptides, polypeptides or proteins having said predetermined property;

(c) isolating the polynucleotide sequence encoding said one or more peptides, polypeptides or proteins having said predetermined property; and

(d) producing said peptide polypeptide or protein.

18. The method of claim 17, wherein step (a) further comprises synthesizing a population of stochastic polynucleotide sequences and translating said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

19. The method of claim 17, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences and translating said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

20. The method of claim 18 or 19, further comprising amplification of said population of stochastic polynucleotide sequences.

21. The method of claim 17, wherein step (a) further comprises synthesizing a population of stochastic polynucleotide sequences and expressing said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

22. The method of claim 17, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences and expressing said population of stochastic polynucleotide sequences to produce said population of peptides, polypeptides or proteins.

23. The method of claim 17, further comprising isolating the polynucleotide sequence encoding said one or more peptides, polypeptides or proteins having said predetermined property.

24. The method of claim 17, wherein said producing in step (d) further comprises chemical synthesis or recombinant expression.

25. The method of claim 17, wherein said predetermined property comprises binding or chemical catalysis.

26. The method of claim 25, further comprising improving said predetermined property by in vitro or in vivo mutagenesis.

27. The method of claim 25, wherein said binding further comprises modification of a biological or chemical property of a compound bound by said peptide, polypeptide or protein.

28. The method of claim 25, wherein said modification of said biological or chemical property of said compound further comprises stimulating or inhibiting at least one biological function of said compound.

29. A method of producing a stochastic polynucleotide population, comprising synthesizing stochastic polynucleotide sequences.

30. The method of claim 29, further comprising chemical synthesis.

31. The method of claim 29, further comprising enzymatic synthesis.

32. The method of claim 29, further comprising joining oligonucleotide building blocks.

33. The method of claim 29, further comprising cleaving said population of stochastic polynucleotide sequences.

34. The method of claim 33, further comprising ligating said cleaved population of stochastic polynucleotide sequences to produce a new ensemble of stochastic polynucleotide sequences.

35. A method of producing a desired compound, comprising combining a population of peptides, polypeptides or proteins encoded by stochastic polynucleotide sequences with two or more reactant precursors under conditions favorable for said precursors to react, and incubating said population of peptides, polypeptides or proteins with said reactant precursors for sufficient time so as to allow the catalysis of said desired compound.

36. A method of identifying a population of peptides, polypeptides or proteins which catalyze a sequence of chemical reactions, comprising:

(a) combining a population of peptides, polypeptides or proteins encoded by stochastic polynucleotide sequences with two or more reactant precursors under conditions favorable for said precursors to react;

(b) incubating said population of peptides, polypeptides or proteins with said reactant precursors for sufficient time to allow the catalysis of said sequence of chemical reactions, and

(c) determining the presence or absence of a compound produced by said sequence of chemical reactions, the presence of said compound indicating that said population of peptides, polypeptides or proteins can catalyze said sequence of chemical reactions.

37. The method of claim 36, further comprising the steps:

(d) dividing the population of peptides, polypeptides or proteins that can catalyze said sequence of chemical reactions into two or more subpopulations;

(e) repeating steps (a) through (c), and

(f) determining the subpopulation which catalyzes the sequence of chemical reactions.

38. The method of claim 37, wherein steps (d) through (f) are repeated one or more times.

39. The method of claim 6, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^4 different amino acid sequences.

40. The method of claim 6, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^5 different amino acid sequences.

41. The method of claim 6, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^6 different amino acid sequences.

42. The method of claim 6, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^7 different amino acid sequences.

43. The method of claim 6, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^8 different amino acid sequences.

44. The method of claim 17, wherein said population of peptides, polypeptides or proteins comprises greater than about 1×10^4 different amino acid sequences.

45. The method of claim 17, wherein said population of peptides, polypeptides or proteins

comprises greater than about 1 x 10^5 different amino acid sequences.

46. The method of claim 17, wherein said population of peptides, polypeptides or proteins comprises greater than about 1 x 10^6 different amino acid sequences.

47. The method of claim 17, wherein said population of peptides, polypeptides or proteins comprises greater than about 1 x 10^7 different amino acid sequences.

48. The method of claim 17, wherein said population of peptides, polypeptides or proteins comprises greater than about 1 x 10^8 different amino acid sequences.

49. The method of claim 29, wherein said stochastic polynucleotide population comprises greater than about 1 x 10^4 different polynucleotide sequences.

50. The method of claim 29, wherein said stochastic polynucleotide population comprises greater than about 1 x 10^5 different polynucleotide sequences.

51. The method of claim 29, wherein said stochastic polynucleotide population comprises greater than about 1 x 10^6 different polynucleotide sequences.

52. The method of claim 29, wherein said stochastic polynucleotide population comprises greater than about 1 x 10^7 different polynucleotide sequences.

53. The method of claim 29, wherein said stochastic polynucleotide population comprises greater than about 1 x 10^8 different polynucleotide sequences.

5,824,514

1. A process for the production of an expression vector which comprises at least one stochastic sequence of polynucleotides, comprising the steps of:

providing in an appropriate buffer at least three different sequences of oligonucleotides, said oligonucleotides each comprising at least 7 nucleotide residues;

polymerizing said oligonucleotides to form a stochastic sequence of polynucleotides; and

ligating said stochastic sequence of polynucleotides into a linearized expression vector.

2. The process according to claim 1 wherein the oligonucleotides further comprise a heptamer.

3. The process according to claim 1, said process further comprising the steps of:

transforming a competent clone with said ligated expression vector;

culturing said transformed competent clone;

purifying said expression vector from said amplified competent clone;

isolating said cultured stochastic sequence of polynucleotides from said expression vector;

cutting said stochastic sequence of polynucleotides by means of at least one restriction enzyme which corresponds to a specific restriction enzyme site present in said stochastic sequence of polynucleotides;

treating said cut stochastic sequence of polynucleotides with a T4 ligase to create a new ensemble of stochastic sequence of polynucleotides containing a new stochastic sequence of polynucleotides; and

ligating said new ensemble of stochastic sequence of polynucleotides into an expression vector.

4. The process according to claim 2 wherein the heptamers are palindromic.

5. The process according to claim 4 wherein the palindromic heptamers are selected from the group consisting of:

5' XTCGCGA 3';

5' XCTGCAG 3'; and

5' RGGTACC 3';

where X=A, G, C, or T, and R=A or T.

6. The process according to claim 1 wherein the oligonucleotides further comprise an octomer.

7. The process according to claims 1 or 6, said process further comprising the steps of:

transforming a competent clone with said ligated expression vector;

culturing said transformed competent clone;

purifying and isolating said expression vector from said cultured competent clone;

cutting said stochastic sequence of polynucleotides by means of at least one restriction enzyme which corresponds to said specific restriction enzyme site present in said stochastic sequence of polynucleotides;

treating said cut stochastic sequence of polynucleotides with a T4 ligase to create a new ensemble of stochastic sequence of polynucleotides comprising a new stochastic sequence of polynucleotides; and

ligating said new ensemble of stochastic sequence of polynucleotides into an expression vector.

8. The process according to claim 6 wherein the octamers are palindromic.

9. The process according to claim 8 wherein the palindromic octamers are selected from the group consisting of:

5' GGAATTCC 3';

5' GGTCGACC 3';

5' CAAGCTTG 3';

5' CCATATGG 3'; and

5' CATCGATG 3'.

10. The process according to claim 1 wherein the stochastic double stranded DNA fragments are about 160 to 800 base pairs in length.

11. A process for the production of an expression vector capable of producing a transcription product or a translation product comprising at least one stochastic sequence of polynucleotides, comprising the steps of:

linearizing an expression vector;

reacting said linearized expression vector with terminal transferase enzyme in the presence of desired ratios of deoxynucleotide-triphosphates of guanine, cytosine, thymidine, and adenine to form a stochastic polynucleotide sequence at each 3' extremity of said linearized vector;

hybridizing said stochastic polynucleotide sequence at a 3' extremity of said linearized vector; and

synthesizing a second strand from said 3' ends of said hybridized vector by incubating with polymerase.

12. A process for the production of a library of expression vectors capable of producing a transcription product or a translation product, said vectors comprising at least one stochastic sequence of polynucleotides, comprising the steps of:

producing at least one stochastic sequence of polynucleotides;

ligating said stochastic sequence of polynucleotides into an expression vector;

transforming a competent clone with said ligated expression vector;

culturing said transformed clone;

screening and/or selecting said transformed clone in order to isolate a clone expressing a stochastic polynucleotide leading to the synthesis of a transcription product or a translation product;

isolating said selected or screened transformed clone; and

isolating the expression vector cultured in said selected or screened transformed clone so identified.

13. A library of expression vectors capable of producing a transcription product or a translation product, said expression vectors comprising at least one stochastic sequence of polynucleotides, produced in accordance with the process of claim 12.

14. An expression vector produced in accordance with the process of claim 1, 11, 12.

15. The process according to claims 11 or 12 wherein said translation product comprises a product having a desired property and is selected from the group consisting of a peptide, a polypeptide or a protein.

16. The process according to claims 1 or 12 wherein said transcription product comprises a product having a desired property and is selected from the group consisting of a RNA or a DNA.

17. The library of expression vectors according to claim 13 wherein said library comprises stochastic nucleotide sequences encoding for at least 10,000 peptides, polypeptides or proteins.

18. A method of producing a diverse population of vectors comprising:

(a) synthesizing a diverse population of stochastically generated polynucleotide sequences comprising greater than about 1×10^5 different polynucleotide sequences, said method consisting of stochastic copolymerization of double stranded oligonucleotides, copolymerization of the four kinds of nucleotide triphosphates consisting of adenine, cytosine, guaninie and thymine, and chemical synthesis, and

(b) inserting said diverse population of stochastically generated polynucleotide sequences into a population of vectors to form a diverse population of vectors containing stochastically generated polynucleotide sequences.

19. The method of claim 18, which said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1×10^6 different polynucleotide sequences.

20. The method of claim 18, which said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1×10^7 different polynucleotide sequences.

21. The method of claim 18, wherein said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1×10^8 different polynucleotide sequences.

22. The method of claim 18, wherein said inserting further comprises hybridization of complementary ends.

23. The method of claim 18, wherein said inserting further comprises ligation.

24. The method of claim 18, further comprising introducing said diverse population of vectors containing stochastically generated polynucleotide sequences into host cells.

25. The method of claim 18, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences.

26. The method of claim 18, wherein step (b) further comprises digesting the diverse population of vectors with a restriction enzyme having a recognition sequence absent in the expression vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of stochastic polynucleotide sequences.

27. A method of producing a diverse population of vectors, comprising stochastically copolymerizing a diverse population of vectors containing double stranded polynucleotides so as to produce a new population of vectors containing greater than about 1×10^5 different polynucleotide sequences.

28. The method of claim 27, wherein said new population of vectors further comprise greater than about 1×10^6 different polynucleotide sequences.

29. The method of claim 27, which said new population of vectors further comprise greater than about 1×10^7 different polynucleotide sequences.

30. The method of claim 27, wherein said new population of vectors further comprise greater

than about 1 x 10^8 different polynucleotide sequences.

31. The method of claim 27, wherein said stochastic copolymerization is effected by hybridization of complementary ends.

32. The method of claim 27, wherein said stochastic copolymerization is effected by ligation.

33. The method of claim 27, further comprising introducing said diverse population of vectors containing stochastically generated polynucleotide sequences into host cells.

34. The method of claim 27, wherein said double stranded polynucleotides further comprise stochastic polynucleotide sequences.

35. The method of claim 27, wherein said double stranded polynucleotides further comprise at least partially stochastic polynucleotide sequences.

36. The method of claim 27, wherein said diverse population of vectors further comprises two or more diverse populations of vectors.

37. A method of producing a diverse populations of vectors, comprising:

(a) obtaining one or more diverse populations of vectors containing diverse sequences of double stranded polynucleotides;

(b) digesting the one or more diverse populations of vectors with a restriction enzyme, and

(c) stochastically copolymerizing the one or more diverse populations of double stranded polynucleotides so as to produce a new population of greater than about 1 x 10^5 different

polynucleotide sequences.

38. The method of claim 37, wherein said new population of vectors further comprise greater than about 1 x 10^6 different polynucleotide sequences.

39. The method of claim 37, wherein said new population of vectors further comprise greater than about 1 x 10^7 different polynucleotide sequences.

40. The method of claim 37, wherein said new population of vectors further comprise greater than about 1 x 10^8 different polynucleotide sequences.

41. The method of claim 37, wherein said stochastic copolymerization is effected by hybridization of complementary ends.

42. The method of claim 37, wherein said stochastic copolymerization is effected by ligation.

43. The method of claim 37, further comprising introducing said diverse population of vectors containing stochastically generated polynucleotide sequences into host cells.

44. The method of claim 37, wherein said double stranded polynucleotides further comprise stochastic polynucleotide sequences.

45. The method of claim 37, wherein said double stranded polynucleotides further comprise at least partially stochastic polynucleotide sequences.

46. The method of claim 37, wherein said diverse population of vectors further comprises two or more diverse populations of vectors.

1. A process for the production of a transcription product or a translation product, comprising the steps of:

producing a stochastically-generated polynucleotide sequence;

producing a library of expression vectors comprising said stochastic polynucleotide sequence;

transforming or transfecting a competent clone with said library of expression vectors;

amplifying said transformed or transfected competent clone;

screening and/or selecting said transformed or transfected clone in order to isolate a clone expressing a stochastic polynucleotide sequence capable of synthesizing a transcription product or a translation product having a predetermined property; and

isolating said selected or screened transformed clone;

isolating a stochastically generated polynucleotide sequence which encodes the identified transcription product or translation product

using the isolated sequence to produce the transcription product or translation product having the predetermined property.

2. A process for the production of a transcription product or a translation product, comprising the steps of:

producing a diverse population of stochastic polynucleotide sequences;

inserting said stochastic polynucleotide sequences into expression vectors to form a diverse population of expression vectors;

transforming or transfecting competent clones with said diverse population of expression vectors comprising said stochastic polynucleotide sequences;

amplifying said transformed or transfected competent clone;

screening and/or selecting said transformed or transfected clones in order to isolate a clone expressing a stochastic polynucleotide capable of synthesizing a transcription product or a translation product having the predetermined property;

isolating said selected or screened transformed clone;

isolating said stochastic polynucleotide sequence which encodes the identified transcription product or translation product;

using the isolated stochastic polynucleotide sequence so as to produce the transcription product or translation product having the predetermined property.

3. A process for the production of a polynucleotide comprising,

producing in an appropriate buffer a diverse population of stochastic polynucleotide sequences;

inserting said stochastic polynucleotide sequences into vectors to form a diverse population of

vectors;

introducing said diverse population of vectors into host cells in a manner to produce a diverse population of transformed host cells;

producing independent clones of the host cells so produced;

screening and/or selecting said independent clones of the host cells to identify host cells comprising a stochastic polynucleotide sequence having at least one desired property; and

isolating said stochastic polynucleotide sequence from the selected or screened clones of host cells.

4. The process according to claim 3, wherein said stochastic polynucleotide sequence comprises a capacity to specifically bind to a compound.

5. The process according to claim 4, wherein said compound is selected from the group consisting of peptides, polypeptides and proteins.

6. The process according to claim 4, wherein said compound further comprises a compound regulating transcriptional activity or a compound regulating replication activity of DNA.

7. A process according to claim 6, wherein said compound further comprises a regulatory protein controlling the transcription or replication of DNA.

8. A process for the production of an RNA comprising,

producing in an appropriate buffer a diverse population of stochastic polynucleotide sequences;

inserting said stochastic polynucleotide sequences into vectors to form a diverse population of vectors;

introducing said diverse population of vectors into host cells in a manner to produce a diverse population of transformed host cells;

producing independent clones of transformed or transfected host cells;

screening and/or selecting said independent clones of the host cells to identify host cells comprising a stochastic polynucleotide sequence capable of producing RNA having at least one desired property; and

isolating said stochastic polynucleotide sequence from the selected or screened clones of host cells.

9. The process according to claim 8, wherein said RNA further comprises a capacity to specifically bind to a compound.

10. The process according to claim 8, wherein said RNA further comprises the capacity to catalyze a chemical reaction.

11. The process according to claim 8, wherein said RNA further comprises a transfer RNA.

12. The process according to claim 1 or 2 wherein said translation product comprises a product having a desired property and is selected from the group consisting of a peptide, a polypeptide or a protein.

13. The process according to claim 1 or 2 wherein said transcription product comprises a product having a desired property and is selected from the group consisting of a RNA or a DNA.

14. A library of vectors produced by the process of claim 1 wherein said library comprises stochastic nucleotide sequences encoding for at least 10,000 transcription products or translation products.

15. A method of identifying a polynucleotide having a predetermined property, comprising:

(a) producing a population of polynucleotides comprising greater than about 1×10^5 different stochastic polynucleotide sequences;

(b) screening said population of polynucleotides for said predetermined property under conditions which allow detection of one or more polynucleotides having said predetermined property.

16. The method of claim 15, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences.

17. The method of claim 15 or 16, further comprising amplification of said population of stochastic polynucleotide sequences.

18. The method of claim 15 or 16, wherein step (a) further comprises expressing said population of stochastic polynucleotide sequences to produce said population of polynucleotides.

19. The method of claim 15 or 16, wherein said population of polynucleotides comprise DNA.

20. The method of claim 15 or 16, wherein said population polynucleotides comprise RNA.

21. The method of claim 15, further comprising isolating said one or more polynucleotide sequences having said predetermined property.

22. The method of claim 15, wherein said predetermined property comprises binding or chemical catalysis.

23. The method of claim 22, further comprising improving said predetermined property by in vitro or in vivo mutagenesis.

24. The method of claim 22, wherein said binding further comprises modification of a biological or chemical property of a compound bound by said polynucleotide.

25. The method of claim 24, wherein said modification of said biological or chemical property of said compound further comprises stimulating or inhibiting at least one biological function of said compound.

26. The method of claim 15, wherein said population of polynucleotides comprises greater than about 1×10^6 different stochastic polynucleotide sequences.

27. The method of claim 15, wherein said population of polynucleotides comprises greater than about 1×10^7 different stochastic polynucleotide sequences.

28. The method of claim 15, wherein said population of polynucleotides comprises greater than about 1×10^8 different stochastic polynucleotide sequences.

29. A method of identifying a polynucleotide having a binding property to a ligand, comprising:

(a) synthesizing a population of stochastic polynucleotide sequences;

(b) inserting said population of stochastic polynucleotide sequences into a population of vectors to form a population of vectors containing stochastic polynucleotide sequences;

(c) expressing in host cells said population of vectors containing stochastic polynucleotide sequences to produce a diverse population of expressed polynucleotides, and

(d) screening said diverse population of polynucleotides with a ligand under conditions which allow binding and detection one or more polynucleotides having said binding property to said ligand.

30. The method of claim 29, wherein said inserting further comprises hybridization of complementary ends.

31. The method of claim 29, wherein said inserting further comprises ligation.

32. The method of claim 29, therein said population of stochastic polynucleotide sequences are produced by stochastic copolymerization of oligonucleotides or polynucleotides.

33. The method of claim 29, wherein said population of stochastic polynucleotide sequences are produced by copolymerization of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine, Thymine and Uracil.

34. The method of claim 29, wherein said population of stochastic polynucleotide sequences are produced by chemical synthesis.

35. The method of claim 29, wherein said population of stochastic polynucleotide sequences comprise DNA.

36. The method of claim 29, wherein said population of stochastic polynucleotide sequences comprise RNA.

37. The method of claim 29, wherein the vector is a plasmid.

38. The method of claim 29, wherein the vector is viral DNA.

39. The method of claim 29, wherein the vector is a hybrid of plasmid and viral DNA.

40. The method of claim 29, wherein the vector is a phage.

41. The method of claim 29, wherein step (c) further comprises digesting the population of vectors with a restriction enzyme having a recognition sequence absent in the vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of new stochastic polynucleotide sequences.

42. The method of claim 29, wherein said population of stochastic polynucleotide sequences, comprise at least 1×10^4 different polynucleotide sequences.

43. The method of claim 29, wherein said population of stochastic polynucleotide sequences, comprise greater than 1×10^5 different polynucleotide sequences.

44. The method of claim 29, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^6 different polynucleotide sequences.

45. The method of claim 29, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^7 different polynucleotide sequences.

46. The method of claim 29, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^8 different polynucleotide sequences.

47. A method of isolating a polynucleotide having a predetermined property, comprising:

(a) producing a population of polynucleotides comprising greater than 1 x 10^5 different stochastic polynucleotide sequences;

(b) screening said population of stochastic polynucleotide sequences for said predetermined property under conditions which allow detection of one or more polynucleotides having said predetermined property, and

(c) isolating the one or more polynucleotide sequences having said predetermined property.

48. The method of claim 47, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences.

49. The method of claim 47 or 48, further comprising amplification of said population of stochastic polynucleotide sequences.

50. The method of claim 47 or 48, wherein step (a) further comprises expressing said population of stochastic polynucleotide sequences to produce said population of

polynucleotides.

51. The method of claim 47 or 48, wherein said population of polynucleotides comprise DNA.

52. The method of claim 47 or 48, wherein said population of polynucleotides comprise RNA.

53. The method of claim 47, further comprising determining the sequence of said one or more isolated polynucleotide sequences having said predetermined property.

54. The method of claim 47, wherein said predetermined property comprises binding or chemical catalysis.

55. The method of claim 54, further comprising improving said predetermined property by in vitro or in vivo mutagenesis.

56. The method of claim 54, wherein said binding further comprises modification of a biological or chemical property of a compound bound by said polynucleotide.

57. The method of claim 56, wherein said modification of said biological or chemical property of said compound further comprises stimulating or inhibiting at least one biological function of said compound.

58. The method of claim 47, wherein said population of polynucleotides comprises greater than about 1×10^6 different stochastic polynucleotide sequences.

59. The method of claim 47, wherein said population of polynucleotides comprises greater than about 1×10^7 different stochastic polynucleotide sequences.

60. The method of claim 47, wherein said population of polynucleotides comprises greater than about 1 x 10^8 different stochastic polynucleotide sequences.

61. A method of isolating a polynucleotide having a binding property to a ligand, comprising:

(a) synthesizing a population of stochastic polynucleotide sequences;

(b) inserting said population of stochastic polynucleotide sequences into a population of vectors to form a population of vectors containing stochastic polynucleotide sequences;

(c) expressing in host cells said population of vectors containing stochastic polynucleotide sequences to produce a diverse population of expressed polynucleotides, and

(d) screening said diverse population of polynucleotides with a ligand under conditions which allow binding and detection of one or more polynucleotides to said ligand, and

(e) isolating the stochastic polynucleotide sequence or sequences having said binding property to said ligand.

62. The method of claim 61, wherein said inserting further comprises hybridization of complementary ends.

63. The method of claim 61, wherein said inserting further comprises ligation.

64. The method of claim 61, wherein said population of stochastic polynucleotide sequences are produced by stochastic copolymerization of oligonucleotides or polynucleotides.

65. The method of claim 61, wherein said population of stochastic polynucleotide sequences

are produced by copolymerization of nucleotide triphosphates consisting of Adenine, Cytosine, Guanine, Thymine and Uricil.

66. The method of claim 61, wherein said population of stochastic polynucleotide sequences are produced by chemical synthesis.

67. The method of claim 61, wherein said population of stochastic polynucleotide sequences comprise DNA.

68. The method of claim 61, wherein said population of stochastic polynucleotide sequences comprise RNA.

69. The method of claim 61, wherein the vector is a plasmid.

70. The method of claim 61, wherein the vector is viral DNA.

71. The method of claim 61, wherein the vector is a hybrid of plasmid and viral DNA.

72. The method of claim 61, wherein the vector is a phage.

73. The method of claim 61, wherein step (c) further comprises digesting the population of vectors with a restriction enzyme having a recognition sequence absent in the vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of new stochastic polynucleotide sequences.

74. The method of claim 61, wherein said population of stochastic polynucleotide sequences, comprise at least 1×10^4 different polynucleotide sequences.

75. The method of claim 61, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^5 different polynucleotide sequences.

76. The method of claim 61, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^6 different polynucleotide sequences.

77. The method of claim 61, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^7 different polynucleotide sequences.

78. The method of claim 61, wherein said population of stochastic polynucleotide sequences, comprise greater than 1 x 10^8 different polynucleotide sequences.

79. A method of producing a diverse population of polynucleotides, comprising stochastically copolymerizing a population of polynucleotides so as to produce a new population of polynucleotides containing greater than about 1 x 10^5 different polynucleotide sequences.

80. The method of claim 79, wherein said new population of polynucleotides further comprise greater than about 1 x 10^6 different polynucleotide sequences.

81. The method of claim 79, which said new population of polynucleotides further comprise greater than about 1 x 10^7 different polynucleotide sequences.

82. The method of claim 79, wherein said new population of polynucleotides further comprise greater than about 1 x 10^8 different polynucleotide sequences.

83. The method of claim 79, wherein said stochastic copolymerization is effected by hybridization of complementary sequences.

84. The method of claim 79, wherein said stochastic copolymerization is effected by ligation.

85. The method of claim 79, wherein said population of polynucleotides comprise DNA.

86. The method of claim 79, wherein said population of polynucleotides comprise RNA.

87. The method of claim 79, further comprising introducing said new population of polynucleotides into host cells.

88. The method of claim 79, wherein said population of polynucleotides further comprise stochastic polynucleotide sequences.

89. The method of claim 79, wherein said population of polynucleotides further comprise at least partially stochastic polynucleotide sequences.

90. The method of claim 79, wherein said population of polynucleotides further comprises two or more populations of polynucleotides.

91. A method of producing a diverse population of polynucleotides, comprising:

(a) obtaining one or more populations of polynucleotides;

(b) cleaving the one or more populations of polynucleotides, and

(c) stochastically copolymerizing the one or more populations of cleaved polynucleotides so as to produce a new population of greater than about 1×10^5 different polynucleotide sequences.

92. The method of claim 91, wherein said new population of polynucleotides further comprise

greater than about 1 x 10^6 different polynucleotide sequences.

93. The method of claim 91, which said new population of polynucleotides further comprise greater than about 1 x 10^7 different polynucleotide sequences.

94. The method of claim 91, wherein said new population of polynucleotides further comprise greater than about 1 x 10^8 different polynucleotide sequences.

95. The method of claim 91, wherein said stochastic copolymerization is effected by hybridization of complementary sequences.

96. The method of claim 91, wherein said stochastic copolymerization is effected by ligation.

97. The method of claim 91, wherein said one or more populations of polynucleotides comprise DNA.

98. The method of claim 91, wherein said one or more populations of polynuclectides comprise RNA.

99. The method of claim 91, further comprising introducing said new population of polynucleotides into host cells.

100. The method of claim 91, wherein said population of polynucleotides further comprise stochastic polynucleotide sequences.

101. The method of claim 91, wherein said population of polynucleotides further comprise at least partially stochastic polynucleotide sequences.

102. The method of claim 91, wherein said population of polynucleotides further comprises two or more populations of polynucleotides.

103. An isolated population of polynucleotides, comprising greater than about 1×10^5 different stochastic polynucleotide sequences.

104. The isolated population of claim 103, further comprising greater than about 1×10^6 different stochastic polynucleotide sequences.

105. The isolated population of claim 103, further comprising greater than about 1×10^7 different stochastic polynucleotide sequences.

106. The isolated population of claim 103, further comprising greater than about 1×10^8 different stochastic polynucleotide sequences.

107. The isolated population of claims 103, 104, 105 or 106, wherein said stochastic polynucleotide sequences comprise DNA or RNA.

5,976,862

1. A process for the production of a host cell capable of producing a transcription product or a translation product comprising an expression vector, wherein said expression vector comprises at least one stochastic sequence of polynucleotides comprising the steps of:

producing a library of expression vectors capable of producing a transcription product or a translation product, said expression vectors comprising at least one stochastic sequence of polynucleotides, said expression vectors being produced by the following steps:

providing in an appropriate buffer at least three different sequences of oligonucleotides, said oligonucleotides each comprising at least 7 nucleotide residues;

polymerizing said oligonucleotides in a manner to form a stochastic sequence of polynucleotides;

ligating said stochastic sequence of polynucleotides into a linearized expression vector; and

transforming a competent host cell with said ligated expression vector.

2. A process for the production of a host cell capable of producing a transcription product or a translation product comprising an expression vector, wherein said expression vector comprises at least one stochastic sequence of polynucleotides comprising the steps of:

producing a library of expression vectors capable of producing a transcription product or a translation product, said expression vectors comprising at least one stochastic sequence of polynucleotides, said expression vectors being produced by the following steps:

linearizing an expression vector;

reacting said linearized expression vector with terminal transferase enzyme in the presence of desired ratios of deoxynucleotide-triphosphates of guanine, cytosine, thymidine, and adenine to form a stochastic polynucleotide sequence at each 3' extremity of said linearized vector;

hybridizing said stochastic polynucleotide sequence at a 3' extremity of said linearized expression vector;

synthesizing a second strand from said 3' ends of said hybridized expression vector by incubating with polymerase; and

transforming a host cell with said expression vector.

3. A process for the production of a host cell capable of producing a transcription product or a translation product comprising an expression vector, wherein said expression vector comprises at least one stochastic sequence of polynucleotides comprising the steps of:

producing a library of expression vectors capable of producing a transcription product or a translation product, said expression vectors comprising at least one stochastic sequence of polynucleotides;

transforming a host cell with said expression vector;

culturing said transformed host cell;

screening and/or selecting said transformed host cell; and

isolating said selected or screened host cell.

4. A host cell produced in accordance with the process of claim 1, 2, or 3.

5. A process for isolating or screening for a host cell clone capable of producing a transcription or translation product comprising:

producing a transformed host cell according to the process of claim 1, 2 or 3;

culturing said transformed host cell;

screening and/or selecting said transformed host cell in order to isolate a specific transformed clone, wherein said specific transformed clone is a host cell whose transformed stochastic sequence is a template for the synthesis of a transcription product or a translation product; and

isolating said selected or screened host cell clone.

6. The process according to claim 1, 2 or 3 wherein said translation product comprises a product having a desired property and is selected from the group consisting of a peptide, a polypeptide or a protein.

7. The process according to claim 1, 2 or 3 wherein said transcription product comprises a product having a desired property and is selected from the group consisting of a RNA or a DNA.

8. The library of expression vectors according to claim 1, 2 or 3 wherein said library comprises stochastic nucleotide sequences encoding for at least 10,000 peptides, polypeptides or proteins.

9. A method of producing a diverse population of host cells comprising:

(a) synthesizing a diverse population of stochastically generated polynucleotide sequences comprising greater than about 1 x 10^5 different polynucleotide sequences, said method consisting of stochastic copolymerization of double stranded oligonucleotides, copolymerizaton of the four kinds of nucleotide triphosphates consisting of adenine, cytosine, guaninie and thymine, and chemical synthesis, and

(b) inserting said diverse population of stochastically generated polynucleotide sequences into a population of vectors to form a diverse population of vectors containing stochastically generated polynucleotide sequences; and

(c) inserting said diverse population of vectors into host cells.

10. The method of claim 9, which said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1 x 10^6 different polynucleotide sequences.

11. The method of claim 9, which said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1 x 10^7 different polynucleotide sequences.

12. The method of claim 9, wherein said diverse population of stochastically generated polynucleotide sequences, further comprise greater than about 1 x 10^8 different polynucleotide sequences.

13. The method of claim 9, wherein said inserting further comprises hybridization of

complementary ends.

14. The method of claim 9, wherein said inserting further comprises ligation.

15. The method of claim 9, wherein step (a) further comprises synthesizing a population of at least partially stochastic polynucleotide sequences.

16. The method of claim 9, wherein step (b) further comprises digesting the diverse population of vectors with a restriction enzyme having a recognition sequence absent in the expression vector and reinserting the digested products into said digested population of vectors to form a different population having a greater number of stochastic polynucleotide sequences.

17. A method of producing a diverse population of host cells, comprising stochastically copolymerizing a diverse population of vectors containing double stranded polynucleotides so as to produce a new population of vectors containing greater than about 1×10^5 different polynucleotide sequences and inserting said new population of vectors into host cells.

18. The method of claim 17, wherein said new population of vectors further comprise greater than about 1×10^6 different polynucleotide sequences.

19. The method of claim 17, which said new population of vectors further comprise greater than about 1×10^7 different polynucleotide sequences.

20. The method of claim 17, wherein said new population of vectors further comprise greater than about 1×10^8 different polynucleotide sequences.

21. The method of claim 17, wherein said stochastic copolymerization is effected by hybridization of complementary ends.

22. The method of claim 17, wherein said stochastic copolymerization is effected by ligation.

23. The method of claim 17, wherein said double stranded polynucleotides further comprise stochastic polynucleotide sequences.

24. The method of claim 17, wherein said double stranded polynucleotides further comprise at least partially stochastic polynucleotide sequences.

25. The method of claim 17, wherein said diverse population of vectors further comprises two or more diverse populations of vectors.

26. A method of producing a diverse populations of host cells, comprising:

(a) obtaining one or more diverse populations of vectors containing diverse sequences of double stranded polynucleotides;

(b) digesting the one or more diverse populations of vectors with a restriction enzyme, and

(c) stochastically copolymerizing the one or more diverse populations of double stranded polynucleotides so as to produce a new population of greater than about 1×10^5 different polynucleotide sequences; and

(d) inserting said new population of polynucleotides into host cells.

27. The method of claim 26, wherein said new population of vectors further comprise greater than about 1×10^6 different polynucleotide sequences.

28. The method of claim 26, wherein said new population of vectors further comprise greater than about 1 x 10^7 different polynucleotide sequences.

29. The method of claim 26, wherein said new population of vectors further comprise greater than about 1 x 10^8 different polynucleotide sequences.

30. The method of claim 26, wherein said stochastic copolymerization is effected by hybridization of complementary ends.

31. The method of claim 26, wherein said stochastic copolymerization is effected by ligation.

32. The method of claim 26, wherein said double stranded polynucleotides further comprise stochastic polynucleotide sequences.

33. The method of claim 26, wherein said double stranded polynucleotides further comprise at least partially stochastic polynucleotide sequences.

34. The method of claim 26, wherein said diverse population of vectors further comprises two or more diverse populations of vectors.

APPENDIX C

Claim 1 of Kauffman 5,723,323	Claim 28 of present Horwitz application
1. A method of identifying a peptide, polypeptide or protein having a binding property to a ligand, comprising:	28. A method of identifying a functional nucleotide sequence which provides a desired biological activity comprising:
(a) providing a ligand for detecting said binding property;	a. providing a means for detecting said desired biological activity;
(b) synthesizing a diverse population of stochastically generated polynucleotide sequences;	b. synthesizing by enzymatic or chemical synthesis a mixed population of nucleotide sequences, wherein said nucleotide sequences comprise an oligonucleotide consisting of a 5' randomized, a central preselected sequence and a 3' randomized sequence, and wherein said randomized sequences are synthesized without reference to a wild type sequence;
(c) inserting said diverse population of stochastically generated polynucleotide sequences into a population of expression vectors to form a diverse population of expression vectors containing stochastically generated polynucleotide sequences;	c. introducing a plurality of the nucleotide sequences into a population of cloning vectors to obtain a plurality of cloning vectors containing the nucleotide sequences;
(d) expressing in host cells said diverse population of expression vectors containing stochastically generated polynucleotide sequences to produce a diverse population of peptides, polypeptides or proteins; and	d. introducing said cloning vectors into suitable host cells; e. expressing said cloning vectors in said host cells; and

(e) screening said diverse population of peptides, polypeptides or proteins with said ligand under conditions which allow binding and detection of one or more peptides, polypeptides or proteins having said predetermined property.	f. screening said host cells using said means for detecting the desired biological activity under conditions which allow detection of one or more host cells comprising vectors which comprise a functional nucleotide sequence which provides the desired biological activity.